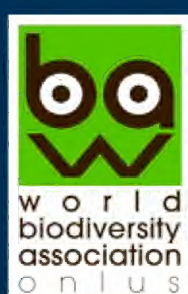


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Cochlodina (P.) kuesteri kuesteri (Rossmässler, 1836) - Posada, Nuoro (Sardinia, Italy)

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***Cochlodina (Procochlodina) kuesteri kuesteri* (Rossmässler, 1836) (Gastropoda Clausiliidae).** Italian endemic species, present in Sardinia and in Gorgona Island (Tuscan Archipelago). In Italy it belongs to the same subgenus *C. bidens* (Linnaeus, 1758) = *incisa* Kuster, 1876, endemic, with limited distribution to Liguria and Emilian Apennines, while a similar is reported in Corsica: *C. meisneriana* (Shuttleworth, 1843). *Cochlodina kuesteri* is present in almost all Sardinia with several populations showing high variability in morphology and shell colour. Populations living in North-Sardinia are referred to as a different subspecies: *C. kuesteri sassariensis* Nordsieck, 1969. The specimen illustrated in the cover photo belongs to the population present in Posada (Nuoro), in the northeast of Sardinia, characterized by a shell blackish-gray in colour with robust and evident ribs on the surface. *Cochlodina kuesteri* is xeroresistant and calcicolous; it lives on calcareous rocks and under stones in stony soils, sometimes even found on ruins and old walls.

Alessandro Margelli. Via Cerretti 77, 56020 Santa Maria a Monte, Pisa, Italy; e-mail: margelli51@gmail.com (photos by A. Margelli)



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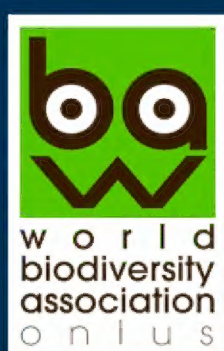
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MONOGRAPH

Paolo Stara & Enrico Borghi

Revision of the genus *Amphiope*
L. Agassiz, 1840 (Echinoidea
Astriclypeidae) with the description of a
new species from the Miocene of France



Amphiope bioculata (Des Moulins, 1837) - Lespignan, France

Revision of the genus *Amphiope* L. Agassiz, 1840 (Echinoidea Astriclypeidae) with the description of a new species from the Miocene of France

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ABSTRACT

The taxonomy of *Amphiope* L. Agassiz, 1840 (Echinoidea, Astriclypeidae), an echinoid distributed in the Oligo-Miocene of Central and Southern Europe, is largely unresolved since the description of most species attributed to this genus was based only on the external morphological features, while important characters, such as the oral plating and the internal support system, were poorly illustrated or completely omitted. Additionally, the type material of some species was missing or badly preserved and geographical/stratigraphical information about the type-locality was unclear. This was the case also for *Amphiope bioculata* (Des Moulins, 1837), the type species of the genus. The poor definition of the earlier described species of *Amphiope* prevented comparison with fossils from other localities and ages, subsequently attributed to this genus. A large part of the earlier species of *Amphiope*, key-taxa for the resolution of the complex taxonomy of this genus, are herein revised by modern methods. For this purpose, the type material available in public institutions has been re-examined and, when possible, new topo-typic material has been collected. As a result, the morphological description of *A. bioculata* has been completed based on fossils from the Middle Miocene of Hérault (France), which is here considered as the type area. The redefinition of the type species allowed to extend comparison and taxonomic discussion to other species earlier attributed to *Amphiope*. Seventeen species are herein confirmed as valid and maintained in the genus *Amphiope*. Three additional species so far attributed to *Amphiope* have been transferred to the genus *Paraamphiope* Stara et Sanciù, 2014: *P. agassizi* (Des Moulins in Cotteau, 1865), *P. cherichirensis* (Thomas et Gauthier, 1889) and *P. baquiei* (Lambert, 1907). *Amphiope boulei* Cotteau, 1914, has been assigned to the genus *Sculpsitechinus* Stara et Sanciù, 2014. *Amphiope romani* n. sp. is described on the basis of a sample from the Serravallian-Tortonian of Touraine (France); it is distinguished mainly by the periproct very close to the posterior margin and the lack of sinus in correspondence of the frontal ambulacra.

KEY WORDS

Echinodermata; Astriclypeidae; *Amphiope*; Neogene; western Atlantic and Mediterranean.

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INTRODUCTION

The earliest records of “*Amphiope*” L. Agassiz, 1840 (Echinoidea, Astriclypeidae) date back to the

late Oligocene of western France (Des Moulins, 1837; Cotteau, 1858-1880; Tournouer, 1870) and the Proto-Mediterranean area (Agassiz L. & Desor, 1847; Des Gras, 1849; Cotteau, 1877, 1895; Pomel,

1883, 1885, 1887; Lambert, 1907, 1910, 1912, 1915, 1927, 1928; Lovisato, 1911, 1914; Cottreau, 1914; Lecointre, 1952; Comaschi Caria, 1955, 1972; Llompert, 1983; Philippe, 1998b; Stara et al., 2012). This genus diffused above all during the Miocene, towards the Atlantic coast of Africa (de Loriol, 1905; Néraudeau & Masrour, 2008), the Atlantic coast of Europe (de Loriol, 1896; Pereira, 2010) and, through the Paratethys and the Middle East (Laube, 1871; Fuchs, 1882; Ali, 1998; Nebelsick & Kroh, 2002), to north-western India (Smith & Kroh, 2011). It went extinct at the end of the Miocene or during the Pliocene. Indeed, a sole specimen was reported from the Pliocene of Algeria by Aymé & Roman (1954).

The taxonomy of *Amphiope* is largely unresolved since this genus accounts for more than 40 species, most of which are nominal species in need of revision due to high intraspecific variation and poor species definition (Smith & Kroh, 2011). Separation between species has been traditionally based on external morphological features, mainly test outline, size and shape of lunules and petals. Important features for species-level taxonomy in the astriclypeids, such as oral plating and internal test support system, were poorly illustrated or omitted completely. The importance of these characters was emphasised in earlier studies (Darteville, 1953; Durham, 1955; Lohavanijaya, 1965; Mooi, 1989; Ziegler et al., 2015) and in Recent researches concerning *Echinodiscus* Leske, 1778 (Jansen & Mooi, 2011, Stara & Sanciù, 2014) and *Amphiope* (Kroh, 2005; Stara & Borghi, 2014).

Most of the earlier described species of *Amphiope* were represented by scanty or badly preserved material, even single specimens, not taking the intraspecific variability into consideration. In some cases, the original illustration was arbitrarily reconstructed, e.g. in *A. hollandei*, as underlined by Cottreau (1914), *A. depressa* Pomel, 1887 and *A. palpebrata*, Pomel 1887. Therefore, they were not completely documented in terms of their morphology, thus preventing a reliable comparison. This condition led to a substantial uncertainty in the species separation within *Amphiope* and to subjective interpretations: e.g. Philippe (1998a) when studying *Amphiope* from the Rhône Basin (France) attributed almost all of the studied specimens to a sole species, *A. bioculata* (Des Moulins, 1837) the type species of the genus, which was interpreted as a taxon with

a broad morphological variability. As a consequence, most of the specimens of *Amphiope* subsequently described in the literature have been assigned to *A. bioculata* (e.g. Kroh, 2005; Pereira, 2010).

Recent studies (Stara & Borghi, 2014) revealed that, despite a large morphological variability in *Amphiope*, structural differences enabled to distinguish species and, in some cases, to transfer species to other genera (Stara & Sanciù, 2014). Structural characters are not readily visible in the illustration provided by earlier studies and were rarely reported in the original descriptions. The re-examination of the type material has often been the only mean allowing the reassessment of earlier species. Another problem affecting some species described in the 19th and early 20th centuries, including the type species, was the lack of the type material and/or the uncertainty regarding geographical/stratigraphical information on the type-localities. This condition prevented, so far, a reliable taxonomic discussion also in studies carried on by modern methods (e.g. Stara & Borghi, 2014), since comparison with the key-species of *Amphiope*, inadequately illustrated and diagnosed and whose type material and/or type locality were uncertain/unknown, remained uncertain.

The aim of this study was the re-definition, based on morphological and morphometric analyses, with emphasis on the plate patterns and the internal support system, of the type species, *A. bioculata*, and of the other earlier established species of *Amphiope*. For this purpose, it was necessary to restudy the type-material which was available in public institutions and, when possible, to collect new material from the respective type-localities.

Based on the redefinition of the key-species of *Amphiope*, comparison with other taxa subsequently attributed to the genus *Amphiope* has been carried on.

PREVIOUS STUDIES, AN OVERVIEW

The evolution of the interpretation of *Amphiope* and of the species attributed to this genus is synthetically provided in the following, starting from the institution of *Scutella bioculata* Des Moulins, 1837, which was subsequently designated as the type species of this genus (Lambert, 1907). We intentionally

omitted to cite the endless and inconclusive taxonomic discussions reported in the literature and focused on the development of the studies on this genus, including the chronology of the erection of new taxa. Most of the earlier works on *Amphiope* have been provided by French scholars, who studied fossil material from the Oligocene and Miocene of France and other Mediterranean countries (North Africa, Italy, Spain).

Des Moulins, a zoologist from Bordeaux (France), erected *Scutella bioculata* on the basis of figures 5–6 of *Scutella bifora* Lamarck reported in the Encyclopédie méthodique (Bruguières, 1791; Figs. 13, 14) and distinguished two varieties named Var. A (“*foraminibus subrotundis*”) and Var. B (“*foraminibus transversé ovatis*”).

These specimens were subsequently consigned to the Muséum Sciences et Nature of Bordeaux.

The specimens labeled MHNbx 2014.6.317 (syntype from Sure, près de Bollène, Vaucluse, France, ex. Requier [N°55]) and MHNbx 2014.6.189 (syntype from Saucats (Gironde, France) [n°4]) were both considered “type for var. A” by Des Moulins (label and 1837 work).

The specimens labeled MHNbx 2014.6.180.1, syntype from carrière Dambert, Gornac, Gironde, France, ex. Laporte aîné [= senior] [n°118] and an incomplete specimen were considered “type for var. B”. Des Moulins did not provide illustration of those specimens and affirmed that they were too badly preserved to represent a distinct species.

L. Agassiz (1841) established two new genera: *Amphiope* and *Lobophora*. He characterized *Amphiope* by its rounded lunules and provided a doubtful and partial oral plating scheme. He noticed that the arrangement of the plates around the lunules in *Amphiope* was different from that in *Lobophora*, though he did not discuss this particular. He also provided a detailed description and illustration of the species *bioculata*, but based it on a incomplete specimen from the “faluns” of Touraine (L. Agassiz, 1841: pl. 11, figs. 1–3). De Loriol (1901) and Lambert (1907), when examining the mold of that specimen (“Moule 30”), noticed that it was poorly preserved and subjectively restored. The location of the specimen and of its “Moule 30” was never provided; as a matter of fact, they were not recorded in the inventory of the Agassiz collection at Neuchâtel (Jeannet, 1929). This interpretation of the type species was not subsequently accepted (de

Loriol, 1901; Lambert, 1907, 1912a, 1927) since Agassiz’s “neotype” was morphologically different from the type of var. A and its finding locality was very far from the two type-localities indicated by Des Moulins (1837). In the same paper L. Agassiz (1841: pl. 11, figs. 6–10) instituted *A. perspicillata* based on a single specimen from the “*terrains tertiaire*” of Rennes (north-western France).

L. Agassiz & Desor (1847) interpreted *Amphiope* as a subgenus of *Lobophora*, since they believed that the different plating structure around the lunules was not valuable for a distinction at the generic level. In the same work, Desor instituted a new species, *Amphiope elliptica*, from the Miocene of the Rhône Basin (France), but he did not provide illustration.

A. Agassiz (1872–74), revised some genera with oval, elongated radially, lunules and placed *Lobophora* L. Agassiz (1841) in synonymy of *Echinodiscus* Leske (1778) (see *Lobophora truncata* L. Agassiz, 1841 = *Echinodiscus truncatus* (L. Agassiz, 1841), separating it clearly from *Amphiope*.

Cotteau (1865) described *A. agassizi*, from the “calcaire à Astéries” of Gironde (western France). The specific denomination was inspired by a label attached to a specimen examined by Cotteau in Des Moulins’s collection. Cotteau noticed that the plate pattern around the lunules was similar to that in living specimens of *Lobophora truncata* (today *Echinodiscus truncatus*) and *L. bifora* (today *E. bisperforatus*), however he did not provide illustration of that particular feature (Figs. 21, 22) and assigned the new species to the genus *Amphiope*. Also Tournouer (1870) underlined that the lunules of *A. agassizi* were more typical of extant *Echinodiscus* than of *Amphiope*.

Cotteau (1877) erected *Amphiope hollandei* n. sp. from the Burdigalian of Corsica, underlying that the plate pattern around the lunules was characteristic for *Amphiope*. He also suggested that the outline of the lunules, radially or transversely elongated, was important only at the specific level.

Pomel (1887) transferred *A. agassizi* into the genus *Tretodiscus* since “*Amphiope differs from Tretodiscus (Lobophora Agassiz, non Curtis) by its transversely, instead than radially, elongate lunules*”. He suggested that *Tretodiscus* differed from *Amphiope* also by the structure of the lantern. In the same work Pomel assigned four new species to *Amphiope* from the Miocene of Algeria, but he did not

provide the repository of the studied material: *A. palpebrata*, *A. depressa*, *A. villei* and *A. personata*.

Cotteau (1895) instituted *A. lovisatoi* and *A. dessii* on the basis of Miocene fossils from Sardinia. The type material was lost in Cagliari during the Second World War. The type locality of *A. lovisatoi* has been recently rediscovered and new topotypic material collected and studied (Stara & Borghi, 2014).

De Loriol (1902) underlined that the specimen designated by Agassiz as neotype of *Amphiope bioculata* was very poorly preserved and did not correspond to the figures 5–6 of *Scutella bifora* Lamarck reported in the *Encyclopédie méthodique* (Bruguières, 1791). Consequently he based his description on specimens from the “Helvétien” of St. Christol and Chemin de Tuileries, both of them near Lospignan (Hérault), considered as the closest to that illustration.

Fallot (1903) examined the Des Moulins’s collection and noted that all the specimens of Var. A and Var. B were badly preserved (Figs. 15–18). Based on the label attached to two specimens of Var. B, Fallot “wrote: “one of them was from Dambert quarry near Gornac, the other between Violle and Saint-Croix-du-Mont, whereas a number of test fragments are deprived of the indication of the finding locality”. Another label attached to var. B by Des Moulins reported: “assigned to a temporary species, *Amphiope ovalifora*, nob. 4 September 1869”. However, Des Moulins did not published any description or illustration of this taxon, likely because the fossils were badly preserved. Although the denomination *A. ovalifora* was utilized by Benoist (1874) and Degrange-Touzin (1882) for some specimens from the Aquitanian of Lariey and Saint-Croix-du-Mont, respectively, the first description and illustration of this taxon was provided by Fallot (1903). However, the photo published by Fallot points to a different species (as suggested by Fallot himself) which, although morphologically close to *A. ovalifora*, has the periproct very close to the posterior margin. Fallot collected a few well preserved specimens from the “middle Aquitanian” of Cabaron, near Castelvieuil, in the same area of Gornac, leaving them in open nomenclature since they differed from the syntype of Var. B by narrower lunules.

Labrie (1904) confirmed that the original label in Des Moulins collection indicated Gornac as the

type locality: “*La collection de Des Moulins contenait depuis fort longtemps une Amphiope provenant de Dambert, près Gornac (Musée de Bordeaux)*”.

Lambert (1907, 1912a, 1915a, 1927) gave a significant contribution to the knowledge of the genus *Amphiope*. He designated (1907) *A. bioculata* (Des Moulins, 1837) as the type species of the genus and distinguished it from *A. ovalifora* by its roundish instead than transversely elongate lunules. However, when studying large samples from the Rhône Basin (Lambert, 1912a) and Gornac (Lambert, 1915a), the distinction based only on this feature became uncertain. To test the variability of the lunules, Lambert examined some specimens from the two type-localities indicated by Des Moulins (1837), Sure and Bordeaux, and encountered two main problems:

1) No village or locality named Sure was known near Bollène. Lambert (1907) proposed Suze-la-Rousse as a possible alternative. However, the specimens from Suze (MNHN-F- A22694-L 18.458 and A22695-L18.458-261) resulted to be morphologically different from the syntype of *A. bioculata* and were embedded by a different sediment (Lambert, 1912a) (Figs. 19, 20). In agreement with de Loriol (1901), Lambert (1912a) strongly suggested that the specimens from the “Helvétien” of Hérault (France) were the closest to the syntype of Var. A and based his concept of *A. bioculata* on the fossils from that area.

The specimen from Touraine, proposed by Agassiz (1841) as neotype for *A. bioculata*, was assigned by Lambert (1912a) to the variety *turonensis*.

The type-locality of Var. B was not detailed by Des Moulins (1837), who simply indicated “Bordeaux”. Lambert (1915b) indicated Gornac as the type-locality of *A. ovalifora*, thus confirming the opinion of Fallot (1903) and Labrie (1904). Lambert (1912b) studied a sample made of 30 specimens from Gornac concluding that the separation between *A. bioculata* and *A. ovalifora* based only on shape and size of the lunules was uncertain.

Lovisato (1911, 1914) erected three new species from the Miocene of Sardinia: *A. montezemoloi*, *A. calvii* and *A. pallavicinoi*. The type material was lost during the Second World War. *Amphiope montezemoloi* has been recently redefined by Stara & Borghi (2014) who found out the type locality and designated a neotype.

Cottreau (1914: plate 5, figs. 1, 8 and plate 6, figs. 1–11) analyzed the morphological variability present in a large sample from Saint-Christol (Les Tuilières), near Nissan (Hérault). He recognized a sole species and affirmed that the differences in the lunules outline alone were not significant for a distinction at the species level.

Lambert & Thiéry (1925) separated the genus *Amphiope* from *Tretodiscus* and indicated the “*Helvétien*” of Hérault (France) as the type area of *A. bioculata*.

Lambert (1927) instituted a new species, *A. labriei*, from the “*Helvétien*” of Aignan (Gers, north-western France) (Fig. 24).

Mortensen (1948), considered *Amphiope* as a subgenus of *Echinodiscus*.

Although Lovén early in 1874 proposed a method to define the plate position on the test of echinoids and underlined the importance of the plating structure, for almost 180 years the distinction at the species level in *Amphiope* has been based only on external test characters and the taxonomic value of the lunule outline has been debated (see Stara & Fois, 2014 for an overview).

Durham (1955) first utilized the analysis of test scheme, food grooves and growth stages in the ontogenesis in the systematic of clypeasteroids. In particular, he published the oral plating scheme of a specimen (UCMP 33846, Museum of Paleontology of the University of California) attributed to *A. bioculata*. He did not provide the finding locality and the label reported only “Miocene of Europe”; however, the test outline and the oral scheme seem to correspond to some specimens from Nissan (see Fig. 9).

Aymé & Roman (1954) described *A. tipasensis* Aymé et Roman, 1954 from the Pliocene of the Tipasa Province (Algeria), based on a sole specimen. No plating scheme was provided, however the narrow and transversely elongate lunules points to a true *Amphiope*, though the apical disc is not visible.

Philippe (1998a), when studying *Amphiope* from the Rhône Basin (France), attributed almost all the examined specimens from that area and from other Mediterranean localities to *A. bioculata*, subjectively interpreting it as a species with a broad variability range.

Kroh (2005) and Pereira (2010) reported a partial plating scheme of the oral interambulacrum 5 in two specimens of *Amphiope* from the Miocene of Austria and of Portugal, respectively.

Stara & Borghi (2014) introduced the analyses of the plate patterns and of the internal test structure as taxonomic tools useful for species-level taxonomy in *Amphiope*. Five species were identified within the fossil material examined from Sardinia (Italy): *A. lovisatoi* Cotteau, 1895, *A. montezemoloi* Lovisato, 1911, *A. nuragica* (Comaschi Caria, 1955) and two additional species left in open nomenclature. But comparison with key-species of the genus *Amphiope*, whose structural characters were unknown, led to uncertain conclusions. The results of that study indicated that a review, based on those features, of the earlier described species of *Amphiope* was needed to improve the poorly resolved taxonomy of the genus.

Stara & Sanciú (2014) established the new genus *Paraamphiope* Stara et Sanciú, 2014 distinguishing it from *Amphiope* by its axial elongate lunules, separated from the posterior petals by at least 3–4 (rarely 2) couples of plates, by its particular scheme of the oral interambulacrum 5 (see discussion) and by strongly branched food grooves. *Amphiope arcuata* (Fuchs, 1882), from the Miocene of Egypt and Libya, was transferred into the new genus, *A. pedemontana* Airaghi (1901), from the Oligocene of Piedmont and Liguria (northern Italy), was assigned to the genus *Echinodiscus*.

MATERIAL AND METHODS

The material under study, together with that utilized for comparison, consists of 209 specimens from 36 different localities and seven countries (Fig. 1). They have been examined at the Muséum National d’Histoire Naturelle of Paris (MNHN-F), Muséum Sciences et Nature of Bordeaux (MHNbX), Musée Requien of Avignon (MRA), Association Paléontologique of Bordeaux (APBA), Museo di Storia Naturale Aquilegia of Masullas, Sardinia (MAC), Museo di Zoologia ed Ecologia (MZE.UNICA) and Museo di Geologia e Paleontologia “D. Lovisato”, at the Università degli Studi di Cagliari, Sardinia (MDLCA). The specimens attributed by Des Moulins (1837) to *Scutella bioculata* Var. A and Var. B are housed at the MHNbX and the specimen attributed by Durham (1955) to *A. bioculata* is housed at the University of California, Paleontological Museum (UCPM).

The species under study were compared with those from Sardinia, described in Stara & Borghi (2014), and from other localities cited in Stara & Sanciù (2014) and Stara et al. (2015), housed at the Museo di Geologia e Paleontologia “Domenico Lo-visato” (MDLCA) and the Department of Animal

Biology and Ecology, University of Cagliari (MZE.UNICA) and the National History Museum of London (NHMUK).

All the fossil specimens of *Amphiope* studied by Pomel are wanting. After Cleevely (1986), a part of the Pomel collection was purchased by the NHML

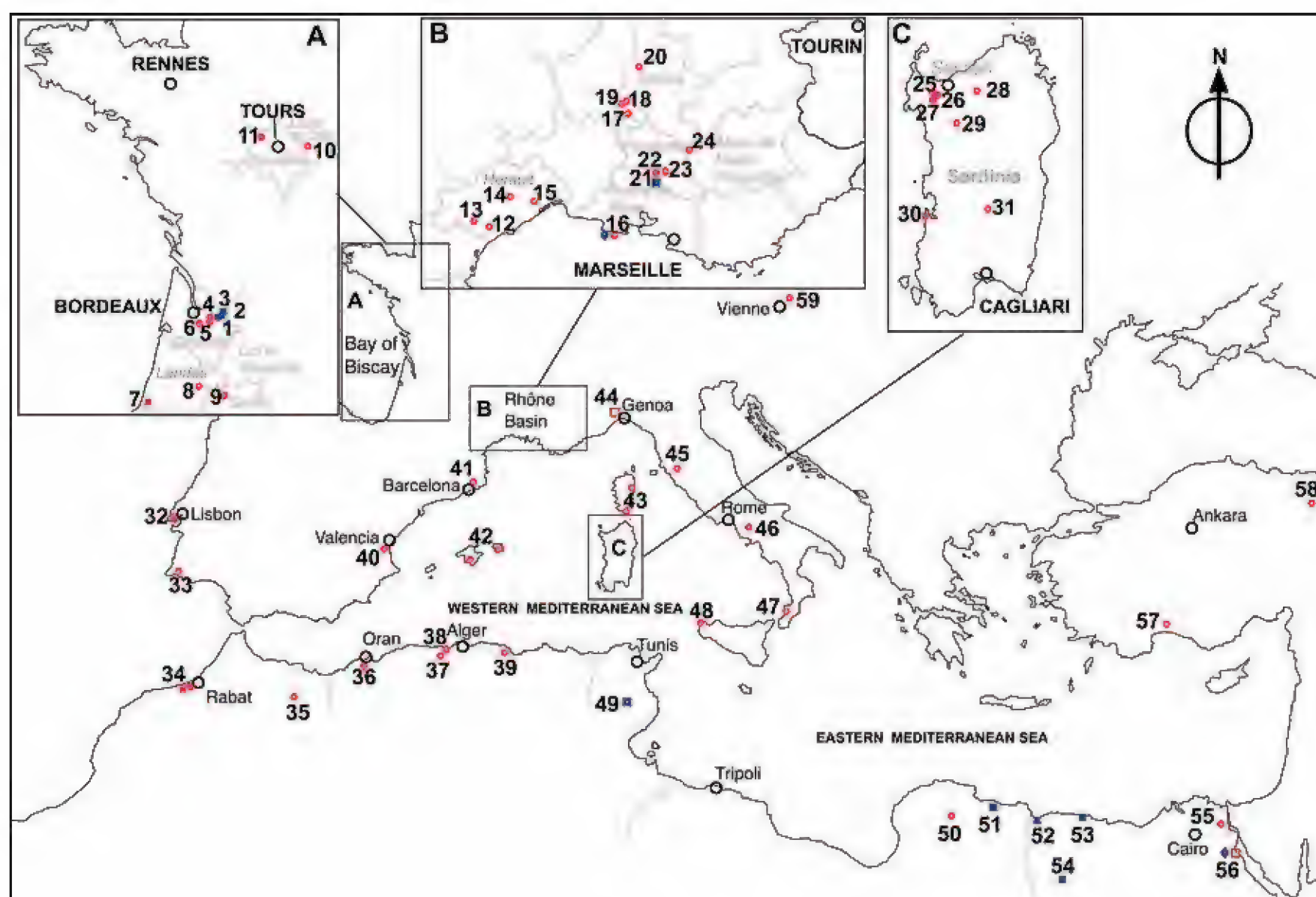


Figure 1. Location map of the findings localities of *Amphiope* and the closely related astriclepeids discussed or cited in this study: box A, western France Atlantic basins; box B, Rhône Basin; box C, Sardinia Basin; D, other localities or basins. ● = *Amphiope*, ■ = *Paraamphiope*, ◆ = *Sculpsitechinus*, □ = *Echinodiscus*. - A, western France, Gironde and other localities: 1=Montségur; 2=La Réole; 3= Pellegrue; 4=Gornac and Saucats; 5= Castellvieil, Cabaron; 6=Léognan (Le Thil); 7=S.t.-Avit; 8=Abesse, S.t-Paul-le-Dax; 9= Montréal; 10=Oisly; 11=Channay sur-Lathan. B, Rhône Basin, France: 12= Nissan-Lespignan; 13= Cruzy; 14=S.t-Felix-de-Lodéne (Lodève); 15=Aspiran; 16=Carry-Le-Rouet; 17=Souze-La-Rousse; 18= S.t-Paul-Trois-Châteaux; 19=S. Restitute; 20=Crest; 21= Cadenet, Vaugines; 22= Cucuron and Blanqui; 23=Cabrière d'Aigues; 24=Châteauneuf-Miravail. C, Sardinian Basin, Italy: 25=La Crucca; 26=Bancali; 27=San Giorgio; 28=Chiaramenti; 29=Bessude and Bonnanaro (all in Sassari Province); 30=Capo Frasca, Arbus; 31=Cuccuru Tuvullau, Nuragus (Cagliari Province). D, other Eastern-Atlantic, Peri-mediterranean and Parathetyan localities/basins. Portugal: 32=Lower Tagus Basin (Lisbon); 33= Algarve. Maroc: 34=Miocene Rabat Basin, Zoubia D'Babei (Helvetian) and Talerhza (upper Aquitanian), respectively; 35=Guercif Basin (Late Miocene). Algeria: 36=Mléta, Oran (Langhian ?); 37=Cherchell (Burdigalian?); 38=Tipasa (Pliocene); 39=Sidi Aïch, Bejaia Province. Spain: 40=Valencia; 41=Barcelona; 42=Balearic Basins. Corse (France): 43= Bonifacio and Aleria (Burdigalian). Italy: 44=Ligurian Oligocene Basin; 45=Manciano, Latium (Middle/? Late Miocene); 46=Campania (Lower-Middle? Miocene); 47=Vibo-Valentia Basin, Calabria (Late Miocene); 48= Case Genna (Marsala, Trapani), Sicily (Langhian-Tortonian). Tunisia: 49=Djebel Cherichira and other nearest localities (Burdigalian). Libya: 50= Sebket El Ghenaïen, Cyrenaica (Miocene); 51=Tobruk area (Middle Miocene). Egypt: 52-54: Western Desert localities: El Salum; Marsa Matruh and Siwa, respectively (Early Miocene); 55-56: Western Desert localities; Gebel Gharra (Upper Burdigalian) and Hurghada, Red Sea (Plio-Pleistocene), respectively. Turkey: 57-58= Mut ? Basin (Burdigalian-Serravalian) and Herzincan Province Basin (Burdigalian). Austria: 59= Vienna Basin (Miocene).

in 1851 (Woodward, 1904), but it included only fossil vertebrata. Some type specimens acquired by E.D. Cope, a private collector, were doubtfully (Cleevely, 1986) incorporated in the Poirrier collection at the American Museum of Natural History of New York (AMNH). As a matter of fact, no specimen belonging to Pomel is actually present at the AMNH (personal communication B. Hussaini and I. Bajo Campos, May 2014).

Since 1922, the type material of the species of *Amphiope* instituted by Lovisato was housed at the Museo Sardo di Geologia e Paleontologia, Institute of Geology, University of Cagliari (<http://sites.unica.it/geomusei/storia/>). On February 28th 1943, the Museum was hit by the bombardment which destroyed almost 80% of the buildings in Cagliari (Pillola & Zoboli, 2014). Only a small part of the fossil material survived that event and was transferred to Ghilarza in 1943. A recent research by one of us (PS) in the unlabelled material stored at the MDLCA confirmed that the type material of *A. desii* Cotteau, 1895, *A. pallavicinoi* Lovisato, 1914 and *A. calvii* Lovisato, 1914 are wanting, but he was able to find the type of *A. montezemoloi* Lovisato, 1911.

Morphology of *Amphiope*

The scheme of the biometric parameters tradi-

tionally used in the taxonomy of *Amphiope* and in this paper is reported in figure 2. Additionally, we tried to transform also the morphological features described in the literature into morphometric data, in particular the shape and size of the lunules, the size of the petalodium and the position of the periproct, taken from as many specimens as possible to avoid subjective interpretations.

The present revision is based also on the study of the internal test structure and the plating arrangement. Measurements and plating pathways have been taken directly from the type-material or newly collected specimens, when possible, and in a few cases from figures presented in the original descriptions.

Morphological abbreviations

See figure 2: β = angle between the major axis of the two lunules; L1-L2 = lunule length and width, respectively; L3 = distance between the posterior petal-tip and the anterior edge of the corresponding lunule, L4 = distance of the genital pores G2 and G3 from the posterior margin of the test, L5-L6 = length and width of the frontal petal, respectively; L7-L8 = length and width of the anterior paired petals, respectively; L9-L10 = length and width of the posterior petals, respectively; L11 = distance posterior border of the periproct-posterior

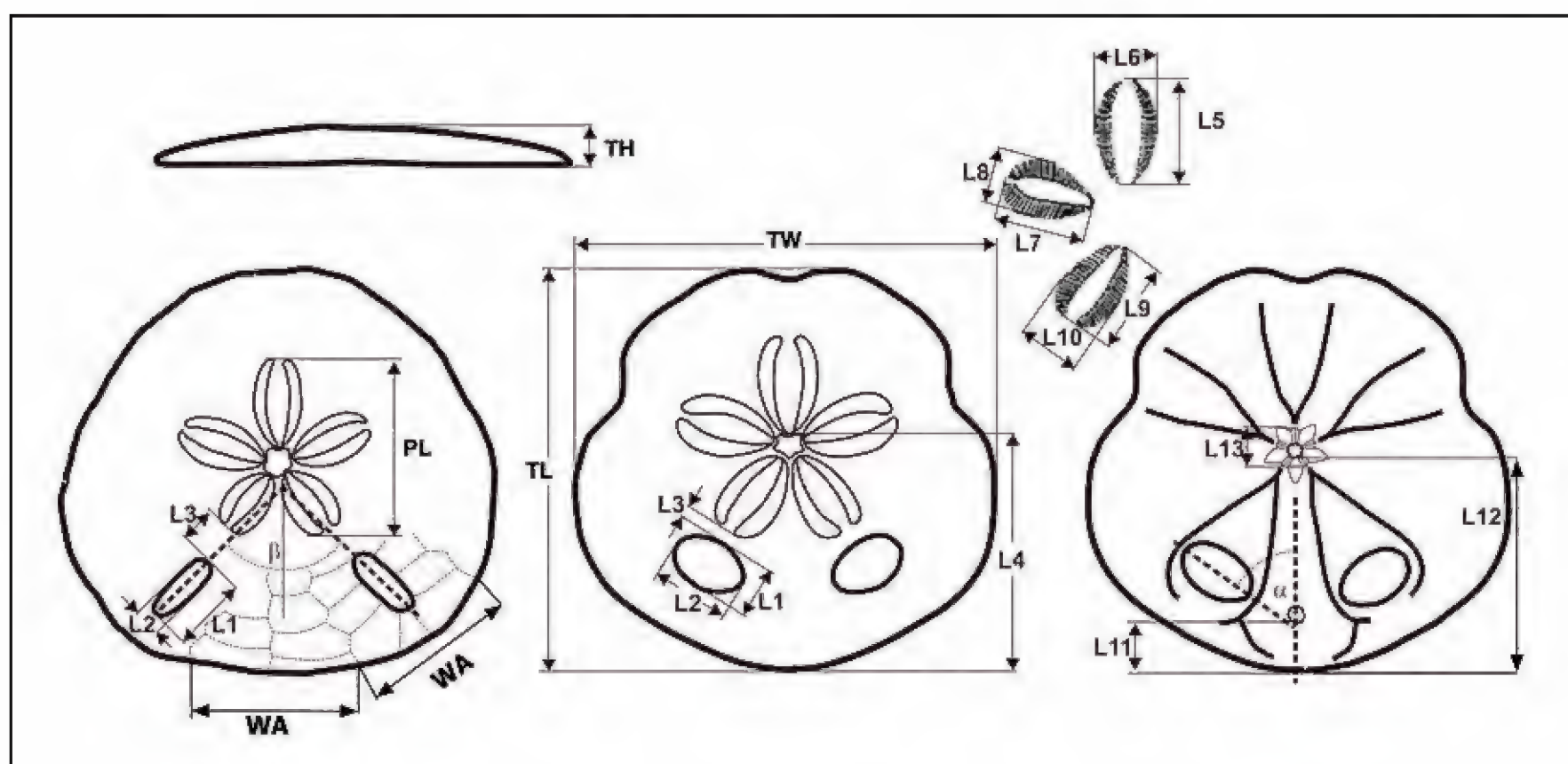


Figure 2. *Amphiope*: scheme of the biometric parameters measured in the studied specimens.

margin of the test; L12 = distance between the posterior border of the peristome and the rear margin, L13 = front-rear diameter of the ambulacral basicoronal circlet. PL = petalodium length; TL-TW-TH = test length, width and height, respectively; WA = width of the interambulacrum 5 at ambitus (measured on the aboral face); \varnothing pc = periproct diameter; \varnothing ps = peristome diameter. TL is expressed in mm, β angle in degree and all the other measurements in % of TL.

Test morphology

Since the range of TL in the material under study was wide (30-170 mm), species are considered "small sized" if maximum TL is below 60 mm, "middle sized" if maximum TL is between 60 and 100 mm, or "large sized" if maximum TL is over 100 mm.

Similarly, the size of the petalodium (PL) in the studied samples of *Amphiope*, *Paraamphiope*, *Echinodiscus* and *Sculpsitechinus* ranges from about 30 up to 60% TL; a petalodium is here assumed to be "small" if PL is below 47% TL, "medium-sized" if PL is between 47 and 54% TL, or "large" if PL is over 54% TL.

To univocally describe the different shape and size of the lunules, the Shape Index (SI) and the Width Index (WI) are introduced. WI equals the area of the rectangle inscribing the lunule [L1xL2 (both measures expressed as % of TL)]. SI is represented by the ratio L2/L1 in *Amphiope* and L1/ L2 in *Paraamphiope* and *Sculpsitechinus*. Due to the relevant variation in the value of WI (55-420), lunules are considered "small" if maximum WI is lower than 100, "mid-sized" if maximum WI is between 100 and 200, or "large" if maximum WI is larger than 200.

Since the lunules are commonly different in the same specimen, the measures of the lunules (L1, L2) were taken from the left lunule, whenever possible. Lunules show different shape and size when viewed from the aboral or the adoral side; additionally, the entrance of the foramen is more or less flared and the walls are inclined. To tackle these problems, measures were taken as indicated in figure 3 (L1). The position of a lunule is univocally indicated by the number of plates in the ambulacral columns separating it from the tip of the corresponding petal.

Food grooves

Since it was not possible to apply a measurement to the grooves, a terminology is introduced to avoid subjective interpretations. The food grooves system consists of five main "trunks" starting slightly after the basicoronal circlet; each trunk soon bifurcates into two main grooves which extend towards the test margin but not reaching it (Fig. 4). The two bifurcations may "develop by a simple scheme" (Fig. 4B-C), i.e. with only a few and short distal secondary grooves, or they are "well branched" (Fig. 4D-E), i.e. with several and rather long "secondary ramifications" which start along the main grooves (Fig. 4E) and from their distal part. The secondary grooves are finer and may spread into smaller branches. The main grooves may be "well marked", i.e. the depressions are rather large and well visible in the oral face (Fig. 4A), or rather "flush" and/or narrow (Fig. 4B). A short branch of each posterior groove surrounds the corresponding lunule and extends towards the periproct.

Plating pathways

The plating pathways were rarely visible on the test surface and only in a few cases we were allowed to prepare the specimens housed in public institutions, to highlight the sutures between plates. Therefore, topotypic specimens were used when possible to improve data and also partial schemes were taken into consideration, since they turned out to be very useful in the taxonomic discussion.

Durham (1955) and Smith (2005) affirmed that the number of plates present in each ambulacral (the extra-petals part) and interambulacral column does not change significantly during growth. Based on this statement, the total number of the plates present in each interambulacral and ambulacral (extra petals) columns and in the space between the petal tips and the corresponding lunules is here used as a taxonomic tool to compare species.

On the other hand, since Durham (1955) noticed that the number of plates bearing pores pairs in the petals progressively increases during growth, comparison between species is carried out based only on the length and width of the petals and the size of the petalodium (PL).

The position of the periproct has been univocally indicated by the plates bounding it, numbered

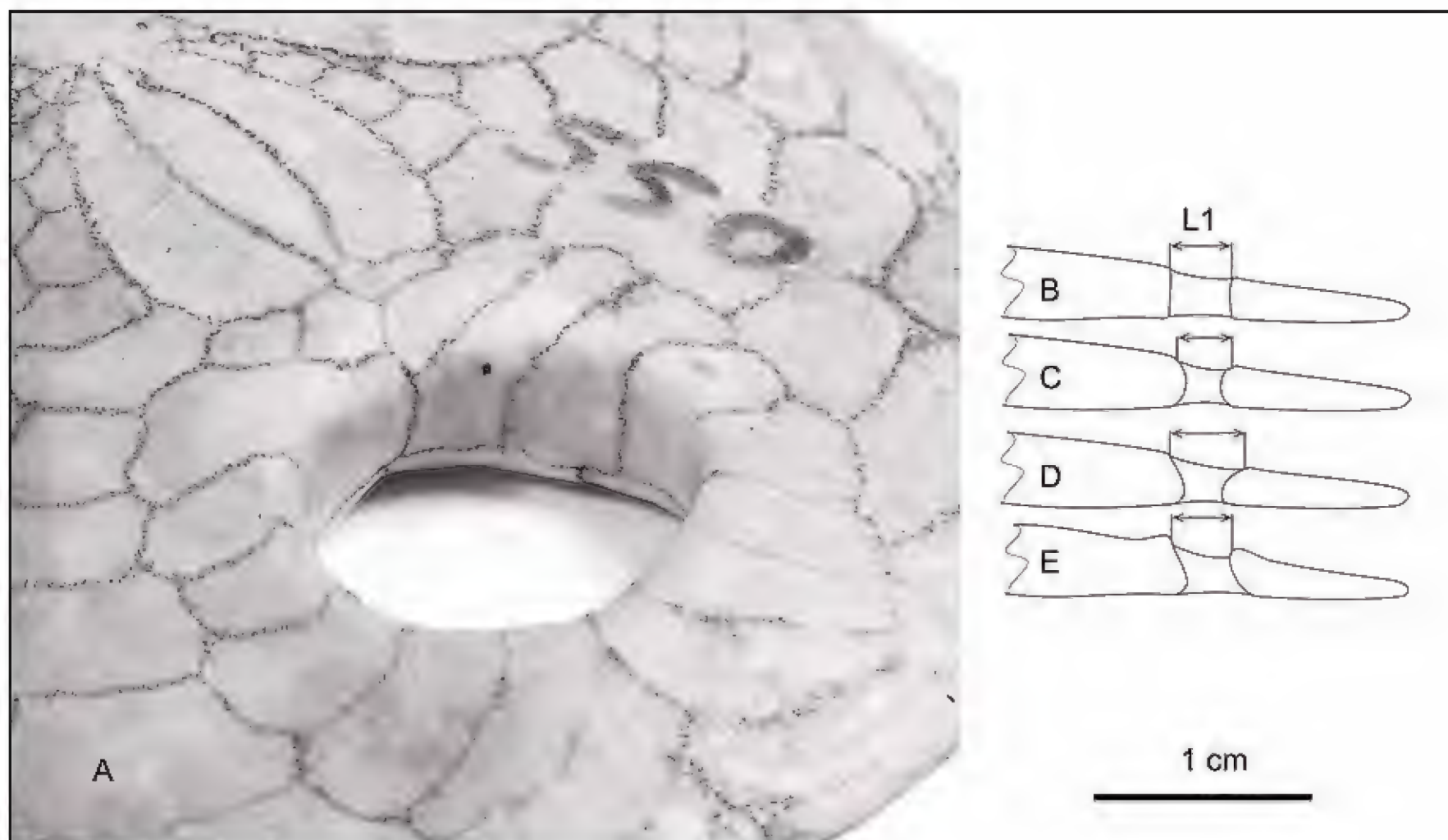


Figure 3. *Amphiope* sp. 2 (MACPL.550), Bancali (Sardinia). A, plating structure of a typical lunule. B-E, schematic sections showing different types of foramen in *Amphiope*: B-C, symmetrical foramen, with perpendicular (B) and convex walls (C), D-E, asymmetrical foramen, with convex walls and much widened (D), with oblique walls (E).

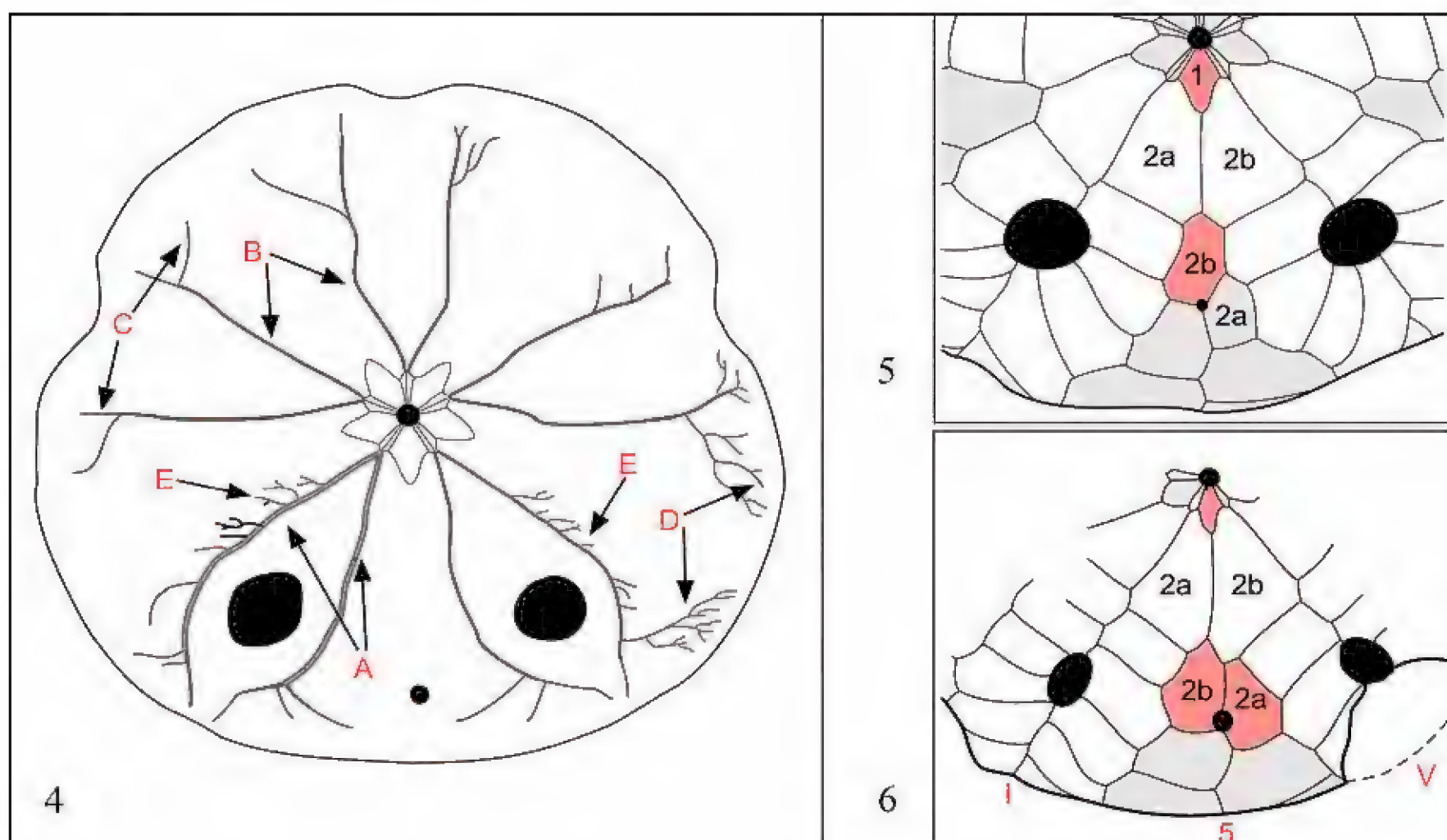


Figure 4. Idealized scheme of the food grooves which can be found in *Amphiope/Paraamphiope*. A, large main trunks; B, thin main trunks; C: simple distal branches; D: complex distal branches; E: additional branches along the main trunks. Figure 5. Adoral scheme of *Amphiope*: in pink plates that characterize the typical arrangement of this genus. Figure 6. Adoral scheme of *Sculpseitechinus*: in pink plates that characterize the typical arrangement of this genus.

after the Lovén's system (Lovén, 1874), in order to avoid the subjective descriptions often reported in the literature, e.g. "far" or "close" from the posterior test margin or the peristome.

In the oral side of almost all of the known species of *Amphiope*, the plates 5.b.2 and 5.a.2 are commonly staggered, with only 5.b.2, which is longer than the others, in contact with both the correspondent ambulacral plates I.a.2 and V.b.2 (Fig. 5). In *Echinodiscus* and in *Sculpsitechinus*, instead, plates 5.b.2 and 5.a.2 are almost paired and both in contact with the correspondent ambulacral plates I.a.2 and V.b.2 (Fig. 6).

Internal test structure

The radiographic analysis has been largely used to study the internal structure of the test, since only in a few cases it was possible to observe sections of the type-material housed in public institutions.

Radiographic photos have always been taken in aboral view, as in all other studies dealing with the scutelliforms (e.g. Ziegler et al., 2015). When possible, specimens of approximately comparable size were chosen for X-Ray imaging. White areas correspond to high-density zones of the test, whereas the darkest areas indicate the presence of internal cavities. The largest dark areas correspond to the central hollow, whose outline may be roundish to sub-pentagonal, and to the radial cavities extending through the interambulacra. The macrocanals running along the interambulacra 2 and 5 contained, respectively, the *caecum* and the *rectum*. The terminology here used to describe the morphological features of the internal support system follows Durham (1955), Mooi (1989) and Mihaljevic et al. (2011). In particular the internal buttressing connecting the lower and upper plated surfaces of the test consists of pillars, which are rounded to oval in cross-section, and bars, connections that are obviously flattened and elongate.

Amphiope bioculata and A. nuragica groups

The systematics follows Kroh & Smith (2010) in general, and Stara & Sanciú (2014) concerning the genera *Paraamphiope* and *Sculpsitechinus*. However, the species under study were divided into two informal groups, as suggested by Stara et al. (2015): the *A. bioculata* group, including the species with

a mean value of $SI \leq 1.6$, and the *A. nuragica* group, with $SI > 1.6$ and more transversely elongate lunules with sub-parallel edges. This grouping likely does not correspond to true phylogenetic lines, however it allowed to limit comparison to the species belonging to the same group, thus making the analysis much easier. Indeed, comparison between all the species studied, indicated other significant differences between the *A. bioculata* and the *A. nuragica* groups.

Type localities

The type localities cited in the literature have been visited, when possible, also to collect new specimens. However, they were often difficult to trace, because old toponyms are often unknown today and some localities, especially when represented by small outcrops, were hidden by natural modifications (e.g. reforestations, landslides) or by changes occurred by the processes starting from the beginning of the '900 (e.g. expansion of towns and villages). Therefore, only a part of the *Amphiope*-bearing localities has been traced and, even in this case, sometimes it was not possible to collect any specimen from them.

The majority of the species attributed to *Amphiope* have been described in the 19th and early 20th centuries and the knowledge about the geology of the *Amphiope*-bearing localities have been greatly improved since then. For example, in earlier studies all the specimens from Saucats were dated to the Aquitanian, while also Burdigalian and Serravallian sediments were recently recognised at that locality (e.g. Londeix, 1991; Nolf & Cahuzac, 2009). A similar situation is known also for Gornac, Nissan and most of the other classic localities of the Aquitaine and Bordeaux basins (Fallot, 1901; Chavanon, 1974; Chauzac et Tourpin, 1999; Chauzac & Janssen, 2010) or of the north-western basins of France (Bouchet et al, 2012).

On the whole, the geographic and the stratigraphic location of the *Amphiope*-bearing outcrops have been updated only when it was possible to trace the localities and geological data were provided by Recent studies. Otherwise, the indication reported in the original labels/descriptions has been used.

Whenever the data concerning the *Amphiope*-bearing deposits were reliable, that was verified on

the field and/or supported by the Recent literature, comparison between species has been carried on based also on the geographical and stratigraphical differences.

DISCUSSION

Redefinition of the type-species, Amphiope bioculata (Des Moulins, 1837)

The specimen (type) assigned by Des Moulins (1837) to *Scutella bioculata* Var. A (MHNbX 2014.6.317) is poorly preserved. It is represented by a whole test with coarse-grained arenite strongly indenting both faces, thus obliterating the main morphological features and the plate structure (Figs. 15, 16). An attempt by C. Laurent (MHNbX) to remove the sediment was not successful. Since “Sure prés Bollène” (Vaucluse), the finding locality indicated by Des Moulins (1837), was unknown, Lambert (1907) and Philippe (1998) suggested that the type-locality was likely Suze-la-Rousse. Two test fragments from Suze-la-Rousse (Drôme) examined in Lambert’s collection (MNHN-F.A22694-L18.458 and MNHN-F.A22694-L18.458-261, Figs. 19, 20) show transversely elongate elliptical lunules (very different from the “foraminibus subrotundaeformis” stated for *A. bioculata*), and are embedded in a brownish fine-grained marly arenite, very different from the pale gray-yellowish arenite indenting the type (Figs. 15, 16). Field research by one of us (PS) confirmed the presence of brownish marly arenite in the surroundings of Suze-la-Rousse (Fig. 1, B17), whereas no pale gray-yellowish arenite was found. Thus, Suze-la-Rousse does not correspond to “Sure prés Bollène”, and the type-locality of *A. bioculata* indicated by Des Moulins remains unknown, as well as the stratigraphic position. De Loriol (1902) and Lambert (1912a, 1915a), based on the study on several populations of *Amphiope* from the Oligo-Miocene of France, indicated the specimens from the “Helvétien” of Hérault as those showing the closest similarity with the syntype of *A. bioculata*. Lambert (1927) even affirmed that “the type from Hérault was replaced by Agassiz (1841) with a specimen from Touraine”. Also Cottreau (1914) based his concept of *A. bioculata* on a sample from the Miocene of Saint Christol (actually Chemin des Tuilières; see Roman, 1974), between Nissan and Lespignan (Hérault).

Following the opinion of de Loriol (1902), Lambert (1912a, 1915a, 1927) and Cottreau (1914), who considered the “Helvétien” of Hérault as the type-area, we based the re-definition of *A. bioculata* on 11 specimens from the Langhian-Serravallian of the surroundings of Nissan and Lespignan. Three of them (MNHN-F.A 57777-9), collected by one of us (PS) from the blue marls of Lespignan (“Marnes bleues inférieures” of Roman, 1974), are consistent with those figured by de Loriol (1901: pl. II, figs. 4, 5) and Cottreau (1914: pl. V, figs. 1-8 and pl. VI, figs. 1-11) and close to the morphological features visible in figs. 5, 6 of the Encyclopédie Méthodique (Bruguières, 1791): middle-sized test (TL=55–74 mm) with rather large (using the old terminology) (WI=56–145) and rounded lunules (SI= about 1), food grooves developing by a simple scheme but well-branched distally. After Roman (1974), the blue marls are dated to the Langhian-Serravallian. Other eight specimens (A2270.L18464a-h) labeled as “Nissan les Tuileries” are close to those from Lespignan, although they probably come from a higher level.

Accordingly to the submission of this article, the authors will submit to the Commission of the ICZN, a request for the establishment of a neotype, based on the sample MNHN-F.A 57777 (Figs. 91, 97). The designation of a neotype is needed since several morphological features of this species, based only on the syntype from “Sure” (MHNbX 2014.6.317), are unclear/unknown thus leading to subjective and controversial interpretation of the type species of *Amphiope*, whose definition is necessary to resolve the complex taxonomy of this genus.

The studied sample shows a morphology as close as possible to the original illustration reported in Bruguières (1791: figs. 5–6); additionally, it enables to take the plating scheme, the main morphometric data and to clear the internal structure. In the oral face, plates 5.a.2 and 5.b.2 are very staggered, with only the plate 5.b.2 in contact with the correspondent post-basical plates I.a.2, V.b.2; the scheme of the oral interambulacrum 5 is variable (Figs. 66–69), but maintains the aforesaid characters. In the aboral face, there are 1–2 couples of plates between the petal tips and the corresponding lunules. In the specimen MNHN-F.A 57777, WA is 36% TL, β is 76°, food grooves develop by a simple scheme, moderately branched distally (Fig. 70, 97).

Distinctive characters of the genus *Amphiope*

Based on the redefinition of the type species, the main diagnostic characters of *Amphiope* described in Smith & Kroh (2011) are here confirmed, with some emendations:

- the lunules in *Amphiope* are commonly rounded or ovoidal and transversely elongate, separated from the petals by only 1–2 (rarely 3) couples of plates;
- food grooves are simple, as well as in the specimen illustrated in figure 5 of the *Encyclopédie Méthodique* (reported in figure 14), or rather branched distally; only exceptionally (*A. sarasini*, see later) they are strongly branched distally and with some secondary ramifications branching off also from the intermediate part of the main grooves;
- in almost all of the known species of *Amphiope*, in the oral side, plates 5.b.2 and 5.a.2 are commonly staggered, with only 5.b.2, which is longer than the others, in contact with both the correspondent ambulacral plates I.a.2 and V.b.2 (Fig. 5). In *Paraamphiope* the plates of the columns “a” and “b”, tend to be parallel to each other along the lunules, while in *Amphiope* the plates of the columns “a” and “b” tend to converge towards the center of the lunules, as we shall see in detail in the following chapters.

Discussion of the earlier species attributed to *Amphiope*

A number of species represented by exhaustive material, that is large samples and/or well preserved specimens, enabled to highlight peculiar distinctive characters for them and to confirm their validity. The description of these well characterised species and the difference between them are reported in detail in the systematics part. In the following, a comparison is carried on between these species and the type-species of the genus, based on the morphological features which are here considered as the most relevant in *Amphiope*.

The visit to Lambert’s collection (MNHN-F), where a large number of specimens of *Amphiope* are stored, highlighted the difficulties encountered by earlier echinologists when comparing samples from different localities and ages using only the external test characters, such as shape and size of test,

petals and lunules. Cottreau (1914) admitted that the distinction at the specific level based only on these features led to subjective interpretations and uncertain results in *Amphiope*. Despite the wide intraspecific variability of this genus underlined in several papers (e.g. Lambert, 1915a, 1927; Cottreau, 1914; Philippe, 1998), Recent studies proved that stable structural differences were present in samples from different localities, allowing a separation at the specific level in *Amphiope* (Stara & Borghi, 2014), as well as in other astriclypeid genera (Stara & Sancier, 2014; Stara & Fois, 2014). Thus, the analysis of the structural characters represents a valuable tool also in the taxonomy of *Amphiope*. Therefore, the present revision, as in our former papers (Stara & Borghi, 2014; Stara & Sancier, 2014), has been based also on morphological biometric analysis and the study of the internal and external test structure.

Plating pathways. The main differences in the plate arrangement between *Amphiope* and the closely related genera, such as *Paraamphiope*, *Sculpsitechinus* and *Echinodiscus* (Figs. 9–12), are found mainly in the oral ambulacra I, V and in the oral interambulacrum 5 (see Stara & Sancier, 2014).

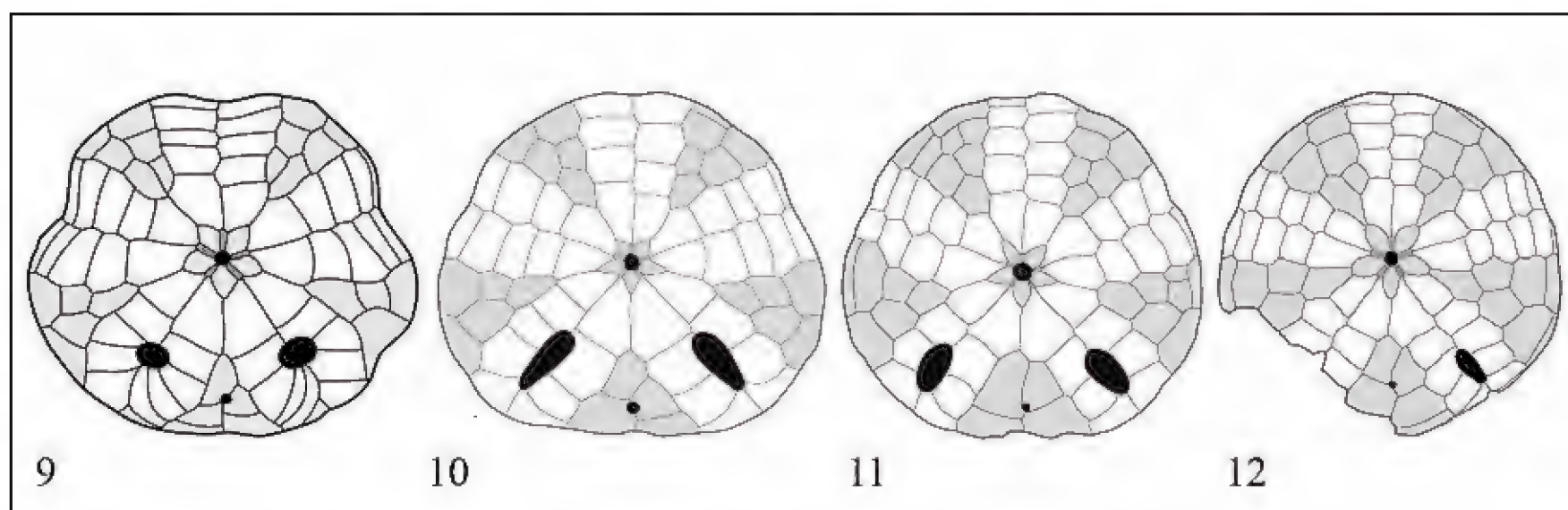
The main differences in the test schemes of *Amphiope* are illustrated for the *A. bioculata* group (Figs. 25–34; Figs. 161, 163, 164) and the *A. nuragica* group (Figs. 35–44; Figs. 151–156).

The variability of the oral structure in the specimens of *A. bioculata* from the Langhian-Serravalian of Lespignan and Nissan is illustrated in figures 66–69: in the oral interambulacrum 5 there are always two post-basicoronal plates in column a (seldom three) and three in column b; plates 5.a.2 and 5.b.2 are staggered, 5.b.2 is always very long, its apex being 27–30% TL far from the posterior test margin; the periproct opens between 5.b.2–5.a.2, 5.a.2–5.b.3, or close to the junction point 5.b.2–5.a.2–5.b.3.

In the *A. bioculata* group, there are not many significant differences between *A. bioculata* (Figs. 27, 28) and *A. elliptica* (late Aquitanian-early Burdigalian of Cruzy; Figs. 25, 26). *Amphiope ludovici* (Tortonian of Blanqui; Figs. 33, 34) has a much lower total number of plates in the interambulacrum 5, when compared to *A. bioculata* and *A. elliptica* (11 against 16) and only two plates are present in columns a and b in the oral interambulacrum 5.



Figures 7, 8: Syntype of *Amphiope bioculata drunensis* (MNHN-F. A22379-18.457) from St.-Paul-Trois-Châteaux. Fig. 7: aboral view, Fig. 8: oral view. The lunules are large ovoidal and rather close to the posterior test margin.

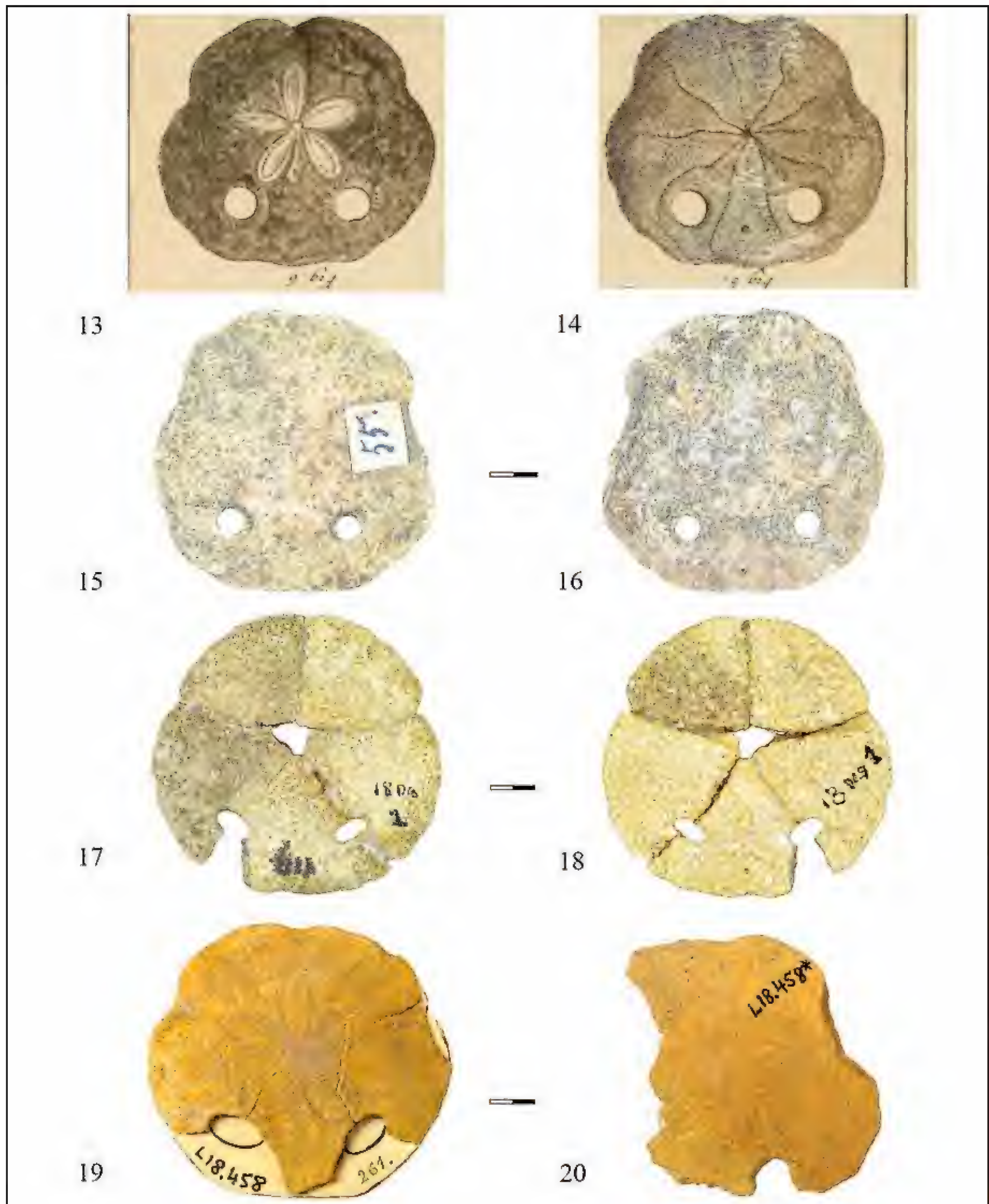


Figures 9–12. Oral plating schemes highlighting the different plate arrangement in the interambulacrum 5 of four genera belonging to the family Astriclypeidae. Fig. 9: *Amphiope bioculata* (UCMP 33846, Miocene, Europe). Fig. 10: *Paraamphiope raimondii* (holotype, MAC.IVM206, Recent, Indonesia). Fig. 11: *Echinodiscus andamanensis* (holotype, PMBC.26346, Recent, Philippines). Fig. 12: *Sculpsitechinus tenuissimus* (neotype, MDL.MAC.IVM207, Recent, Indonesia).

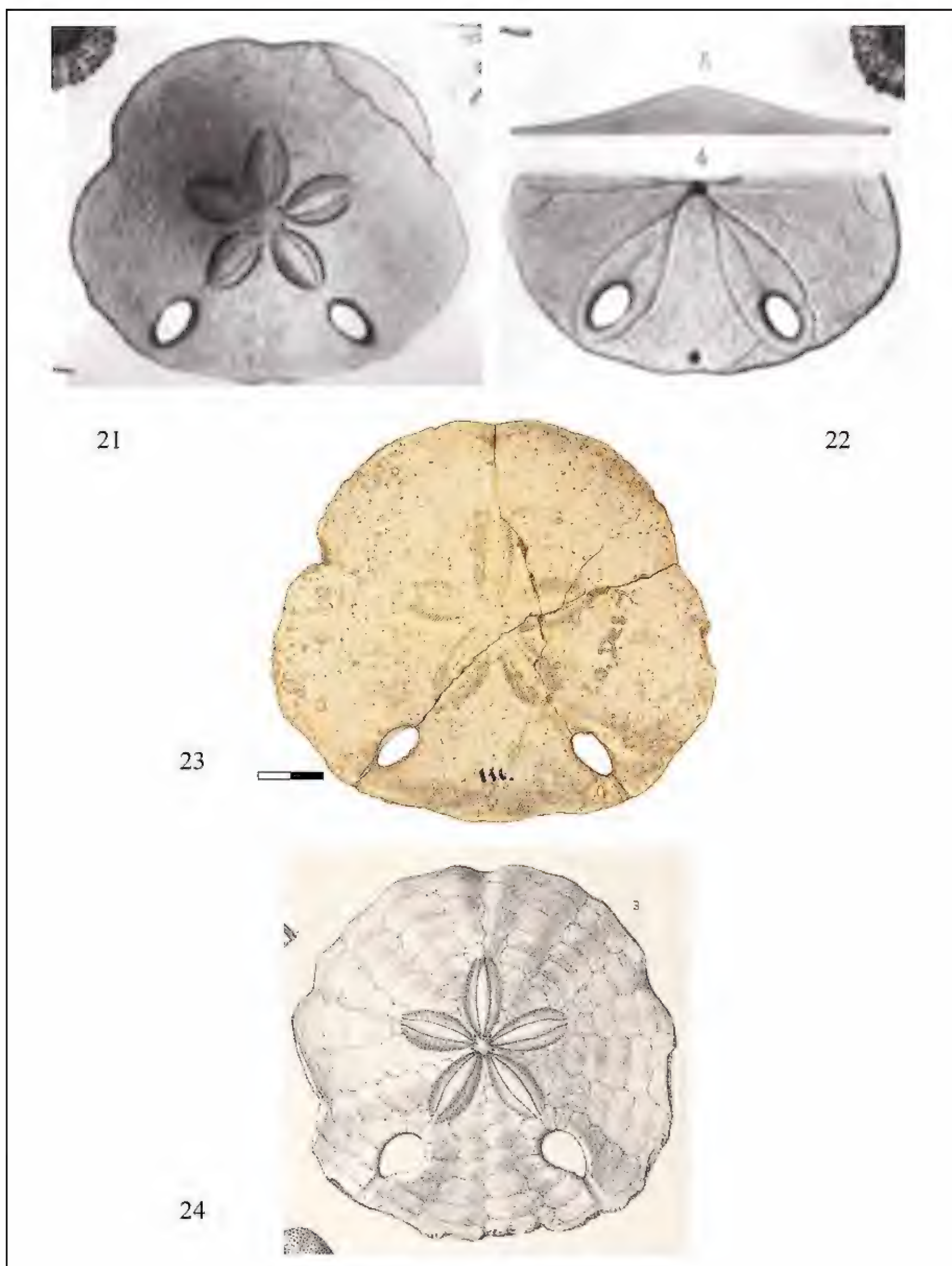
Also *A. lorioli* (Tortonian of St-Félix-de-Lodez; Figs. 31, 32) has a high number of plates (15–16), its petalodium is smaller on the average when compared to the other three species and, as a consequence, the lunules are farther from the posterior test margin.

Within the *A. nuragica* group, in the oral interambulacrum 5 of *A. nuragica* (Chattian-Aquitania; Figs. 35, 36) there are three and four plates in column a and b, respectively; *Amphiope hollandei*

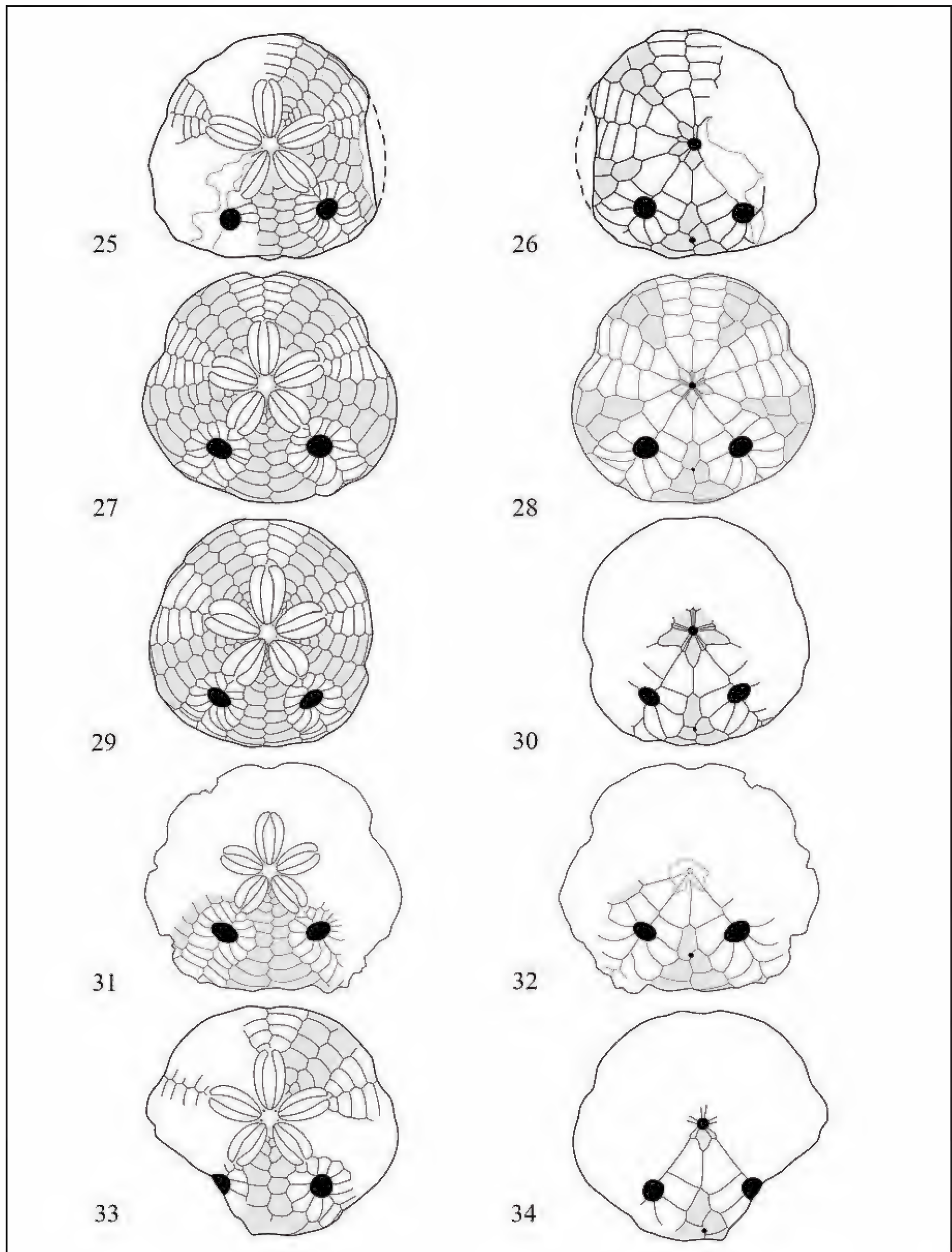
(Burdigalian of Bonifacio, Corse; Figs. 37, 38) has three and three plates; *Amphiope transversifora* (Langhian, Figs. 39, 40) two/three; *Amphiope deyrieri* (Serravallian; Figs. 41, 42) two and two, *A. sarasini* (Serravallian-Tortonian; Figs. 43, 44) two and three, though column “a” is only partially visible in the type (but it is well visible in other specimens, e.g. MNHN-F.A.57788-89). As with regard to the whole number of plates in each column of the interambulacrum 5, *A. nuragica* has up to 20 plates;



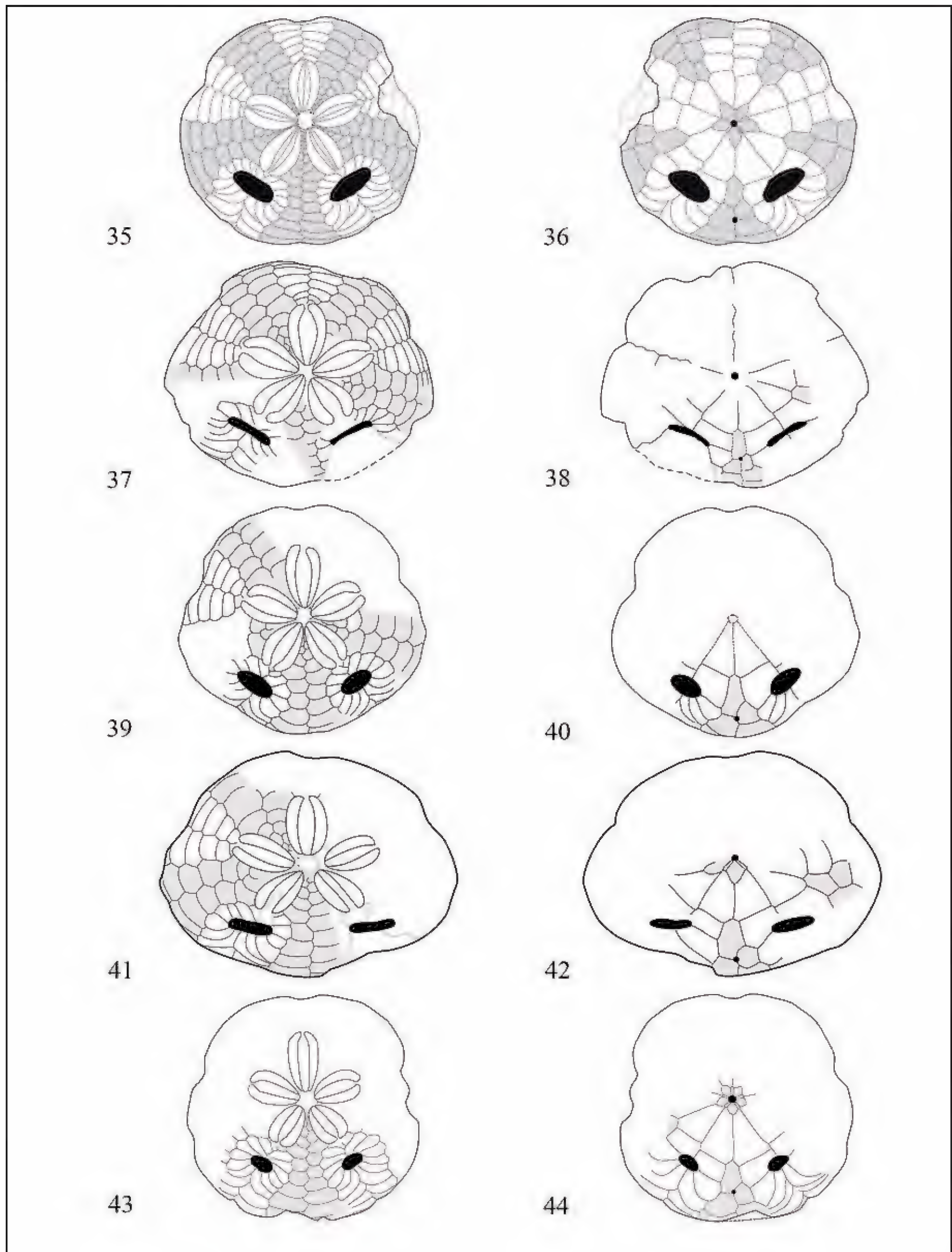
Figures 13–20. *Amphiope bioculata*. Figs. 13, 14: *Scutella bifora* var. c (foraminibus subrotundis) Lamarck, from an unknown locality; Encyclopédie méthodique (Bruguières, 1791; pl. 147, figs. 5, 6); aboral (Fig. 13) and oral (Fig. 14) views. Figs. 15, 16: type (MHNbX 2014.6.317) of *Scutella bioculata* Var. A (foraminibus subrotundis) Des Moulins, 1837, Sure près Bollène, Vaucluse (France), aboral (Fig. 15) and oral (Fig. 16) views. Figs. 17, 18: type (MHNbX 2014.6.180.2) of *Scutella bioculata* Var. B (foraminibus transversé ovatis) Des Moulins, 1837, Gornac, Gironde (France); aboral (Fig. 17) and oral (Fig. 18) views. Figs. 19, 20: aboral view of the specimens from Suze la Rousse (Drôme) indicated doubtfully as A. cfr. *bioculata* by Lambert (1912) (MNHN-F-A L18.458-261; L18.458*); the drawings of missing part of the test and lunule margins (Fig. 19) were originally provided by Lambert.



Figures 21–24. *Paraamphiope agassizi*. Figs. 21–22: specimen from unknown locality figured in Cotteau (1864, pl.14, figs. 3–5), aboral (Fig. 21) and lateral (Fig. 22) and oral views; Fig. 23: aboral view of the syntype [MNHNBx (111) 6-194], from S. Albert. Fig. 24: “*Amphiope*” *labriei* Lambert (1928b).



Figures 25–34. Aboral and oral plating schemes of five species belonging to the *A. bioculata* group. Figs. 25, 26: *A. elliptica* from Carry (MNMN-F.A22706-L18.471). Figs. 27, 28: *A. bioculata* from Lespignan (MNHN-F.A 57777). Figs. 29, 30: *A. ovalifera* from Gornac (MNHN-F.A22710-L18.477c). Figs. 31, 32: *A. lorioli* from St-Félix-de-Lodez (MNHN-F.A22707-L 18472Aa). Figs. 33, 34: *A. ludovici* from Blanqui (MNHN-F.J00999-L18473).



Figures 35–44. Oral and aboral plating schemes of five species belonging to the *A. nuragica* group. Figs. 35, 36: *A. nuragica* from Cuccuru Tuvullau (Sardinia) (MAC.PL1680). Figs. 37, 38: *A. hollandei* from Bonifacio (Corse). Figs. 39, 40: *A. transversifora* from Saint-Paul-Trois-Châteaux (France), syntype. Figs. 41, 42: *A. deyrieri* from Cadenet, Vaucluse (France) (MNHN-F A22705-L18470a). Figs. 43, 44: *A. sarasini* from Cruzy (France) (MNHN-F J00985.L18480).

A. transversifora and *A. deydieri* 11–12 plates, in *A. sarasini* there are 15 plates on the average, more than expected for a relatively Recent species (Serravallian-Tortonian) (see Stara & Borghi, 2014).

The results of a comparison carried out between the plate structure of *A. bioculata* (Langhian-Serravallian of Lespignan, Figs. 27, 28) and some species from the western basins of France, separated from the type species by large geographical and geological distances, are illustrated in figures 157–164: “*Amphiope*” *agassizi* from the Rupelian of Montségur (Figs. 157, 158) and Pellegrue (Fig. 162, only the oral face is available to study in this specimen), *A. ovalifora* (Aquitanian of Gornac, Figs. 29, 30), *A. romani* n. sp. (Serravallian-Tortonian of Channay-sur-Lathan, Figs. 161, 163) and *A. romani* var. *turonensis* (Serravallian of Oisly; Fig. 164, only the oral face). Although “*Amphiope*” *agassizi* is a more ancient taxon, it clearly differs from *A. bioculata* and *A. ovalifora* by the structure of the ambulacra I and V and the interambulacrum 5, which corresponds to the typical plating scheme of *Paraamphiope* (see Stara & Sanciù, 2014), that is plates 5.a.2 and 5.b.2 more paired than in *Amphiope*, the presence of three to five plates (instead of one or two) separating the petal tips from the correspond-

ing lunules (which are radially elongate) and a lower number of plates in the interambulacrum 5. For this reason this species is here transferred to the genus *Paraamphiope*. *Amphiope romani* n. sp. has the lowest number of plates in this group and shows the typical characters of a relatively recent (Late Miocene) *Amphiope*, that is: plates 5.a.2 and 5.b.2 staggered and the presence of two plates between the petals and the corresponding lunules (at least in the specimen reported in figures 161, 163). It shares with “*A.*” *agassizi* the shape of plate 5.b.2., which is very large and not much elongate, thus similar to *Paraamphiope*. This similarity is even closer when comparing *A. romani turonensis* (Fig. 164) and the specimen of “*A.*” *agassizi* from Pellegrue (Fig. 162). However, the plates surrounding the lunules in “*A.*” *agassizi*, are clearly arranged differently, with respect to those of *A. romani* n. sp. In the former the plates of the column “a” tend to be parallel to each other, as well as those of the column “b”. In the later (as in all other *Amphiope*), however, the plates of the columns “a” and “b” around the lunules tend to converge towards the centre of these.

The results of a comparison between “*A.*” *bouleii* (Aquitanian of Carry-le-Rouet) and “*A.*” *baquiei* (Tortonian of Cucuron), the sole two species from



Figures 45–50. Different shapes of lunules in *Amphiope*. Figs. 45, 48: roundish to slightly transversely ovaloid outline, with flared aboral edge. Figs. 46, 49: radially elongate ovaloid lunule, with symmetrical foramen and convex walls. Figs. 47, 50: narrow transversely elongate lunule with symmetrical perpendicular walls.

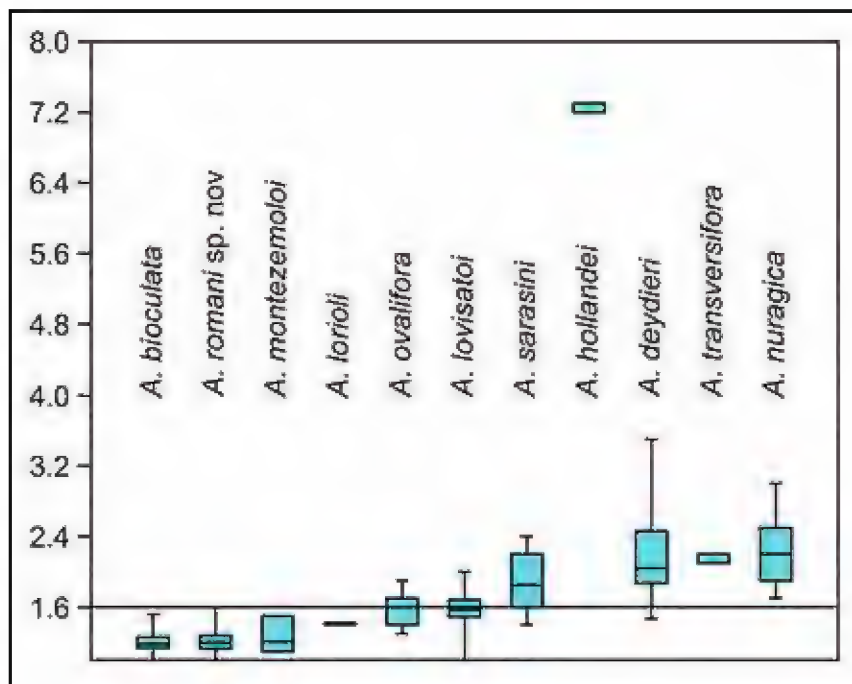


Figure 51. Box-plot showing the mean values and the intra-specific variability range of SI (Shape Index) in different species of *Amphiope*. The horizontal line at SI=1.6 separates two informal groups: The *A. bioculata* group (below the line) and the *A. nuragica* group (above).

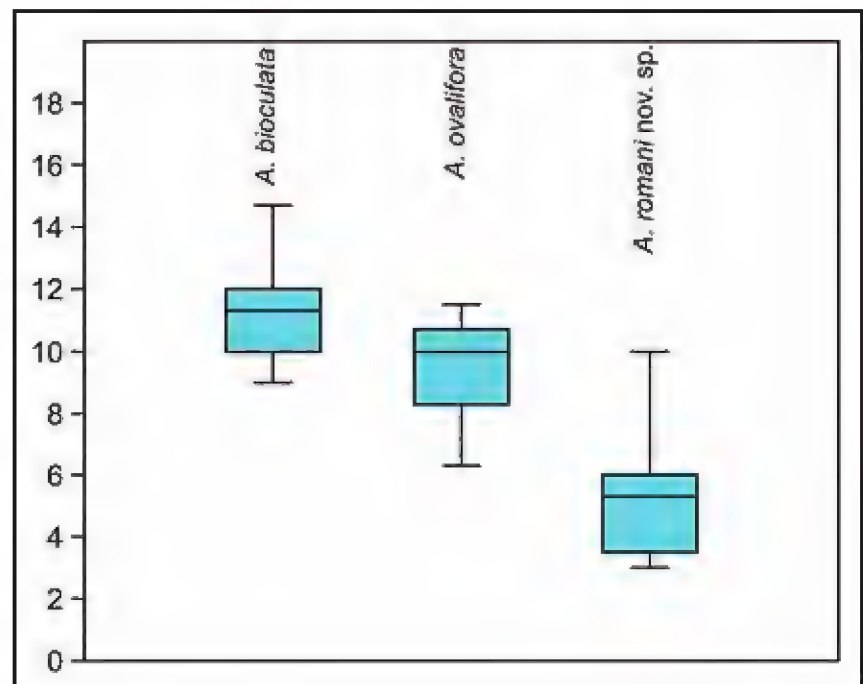


Figure 52. Box-plot comparing the values of L11 (distance of the periproct from the posterior test margin) in three species of *Amphiope*. The number of specimens examined for each species is comparable. Measures are expressed in mm.

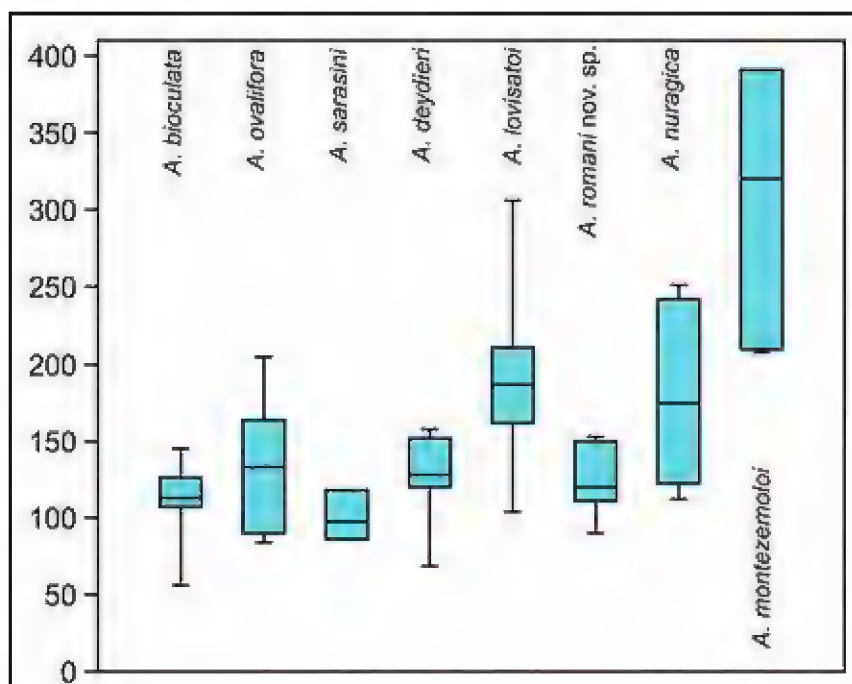


Figure 53. Box-plot showing the intraspecific variability range of WI (Width Index) in different species of *Amphiope*.

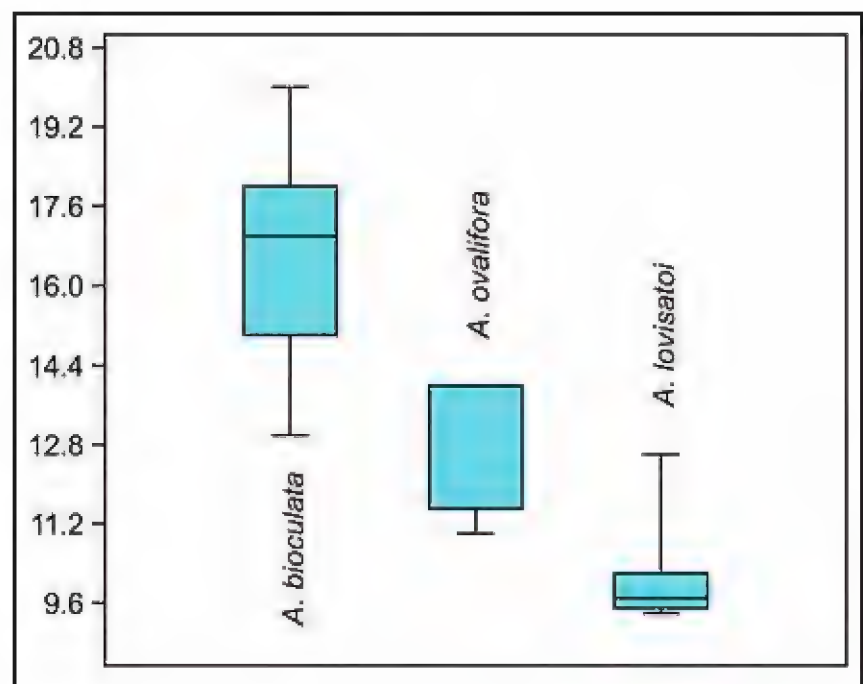


Figure 54. Box-plot comparing the mean value and the variability range of TH (test height), in mm, in some species of *Amphiope* with apparently similar specimens.

the Rhône Basin with radially elongate ovoidal lunules, with *S. tenuissimus* (Recent, Indonesia) and “*A.*” *agassizi* (Rupelian of Pellegrue), are illustrated in figures 55–60. Both “*Amphiope*” *bouleii* and “*Amphiope*” *baquiei* have three and four plates, respectively, between the lunules and the tips of the corresponding petals (Figs. 55, 58), and the first post-basical plates in each column of the oral interambulacrum 5 are paired (Figs. 56, 59). But “*A.*” *bouleii* matches the typical plating scheme of

Sculpsitechinus with only the plate 5.b.2 in contact with both the correspondent ambulacral plates I.a.2 and V.b.2, as in *S. tenuissimus* (Fig. 57), while “*A.*” *baquiei* matches the plating arrangement of *Para-amphiope* (Fig. 60) just as “*A.*” *agassizi*. In “*A.*” *baquiei* the plates 5.a.2 and 5.b.2 are inversely arranged; however similar cases were observed also in *A. nuragica* (Stara & Borghi, 2014) and do not invalidate the comparison.

Although in the studied specimens the lunules

are roundish or slightly radially elongate, Lambert (1907) based “*A. baquiei*” on a specimen from Cucuron with drop-shaped lunules (Fig. 61).

Internal structure. Radiography as a diagnostic tool in *Amphiope* was already used by Darteville (1953), who provided an X-ray illustration of *A. neuparthi*. Stara & Borghi (2014) and Stara & Sanciù (2014) described different kinds of internal structures in *Amphiope* and highlighted relevant differences between *Amphiope* and living *Sculpsitechinus*. An example of the correspondence between the test surface and the main internal structures in *Amphiope* is illustrated in Stara & Sanciù (2014: pl. 2, figs. 1–4: *Amphiope* sp. 3 = *A. romani* n. sp.). The main difference is found in the floor of the central hollow, which in *Amphiope* is strengthened internally by radial low ridges, whereas in *Sculpsitechinus* (directly observed only in living specimens) only a net made by thin trabeculae is present on the floor (see Stara & Fois: 2014, pl. 2, figs. 1–8).

The radiographic analysis may highlight relevant differences in species which are otherwise apparently similar on the basis of the external features. This is the case of *A. elliptica*, *A. bioculata* and *A. ovalifora*, whose plating schemes are similar, as seen above. The radiography of *A. elliptica* (Fig. 76) shows a much more complicated buttress system, with several small pillars and small spaces between elements extending towards the periphery of the test; the main central cavity is subcircular; a part of it is filled with coarse sediments since in this case the test is crashed. Also in *A. bioculata* (Fig. 77, MNHN-F A778) the internal buttress system is complicated, but pillars are stronger and more spaced, the internal cavity is much larger, starring to almost pentagonal (which is here considered as a more evolved character). *Amphiope ovalifora* (Fig. 78) differs clearly from the other two species by its central cavity, which is larger and distinctly sub-pentagonal, the peripheral buttress system, much more developed and densely packed, almost massive, towards the test margin, the macrochannel running along the interambulacrum 4, which is shorter (in this specimen the radial channel leading to the periproct is obliterated by recrystallization processes).

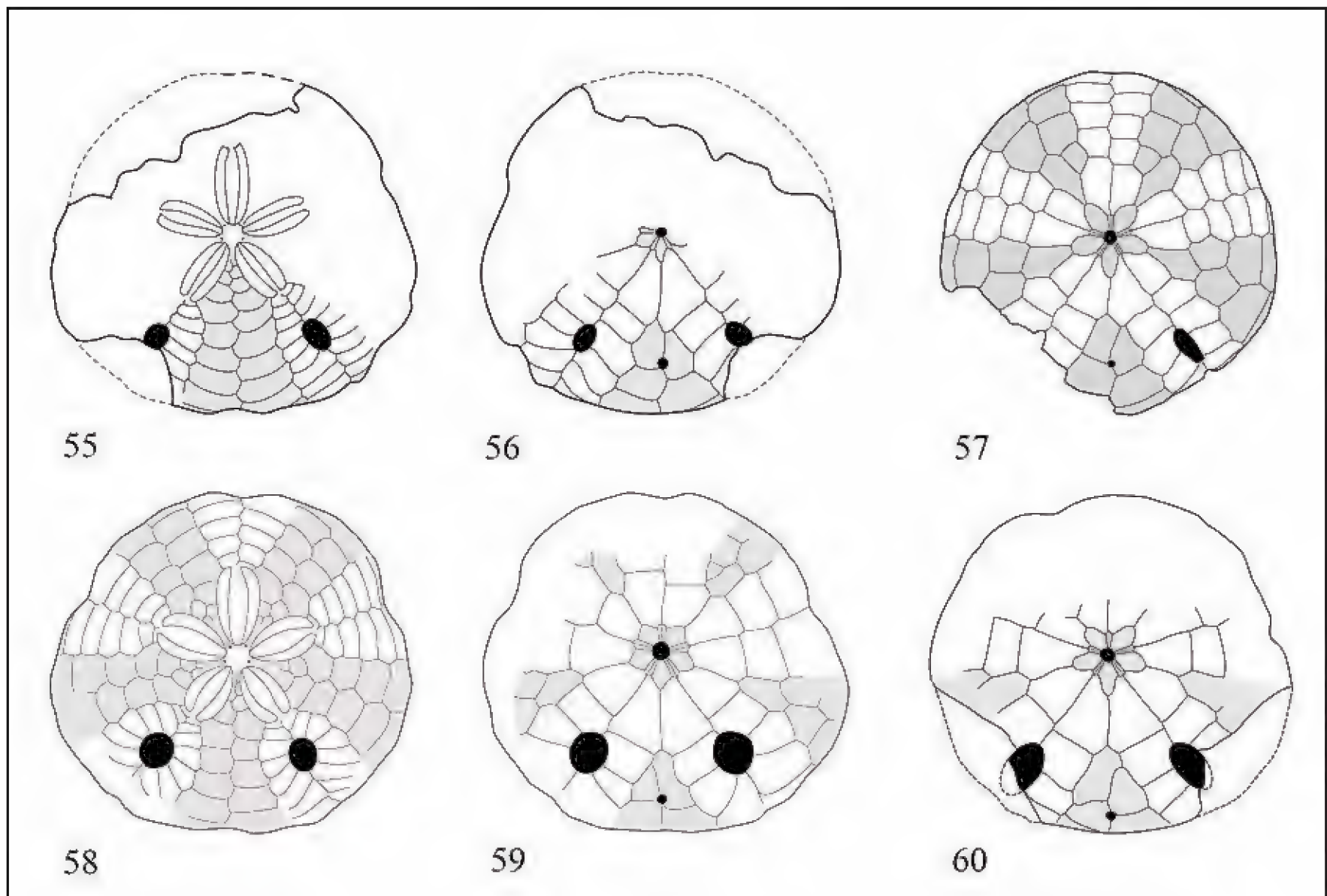
Lunules. Endless discussions occurred between the end of the ‘800 and the beginning of the ‘900

about the relevance of lunules in the taxonomy of *Amphiope* and the closely related genera (see L. Agassiz, 1841; Pomel, 1885; Airaghi, 1901; Lambert, 1915a). Recent studies, such as Stara & Borghi (2014) and Stara & Sanciù (2014), demonstrated the validity of this morphological feature as a tool in species distinction when appropriately used.

Amphiope has two ambulacral lunules, rounded or ovoidal transversely elongate, which are surrounded by two groups of plates, one on each face of the test (Fig. 3A). Plates bounding the lunules are more numerous aborally. The suture between the two groups of plates is linear intra-foraminal, that is located on the wall of the foramen connecting the two test faces (Fig. 3A). This feature distinguishes all known astriclypeids from the mellitids, which have a festooned suture (Mooi, 1989, fig. 30). The lunules show a wide intraspecific variability with regard to shape and size and may differ even in the same specimen. The outline may be wide roundish (Fig. 3A), ovaloid and transversely elongate as in *Amphiope* sp. 2 from Bancali, Sardinia (Figs. 45, 48), or sometimes the lunules can vary from roundish to radially elongate, as in *Amphiope* sp. from Capo Frasca, Sardinia (Figs. 46, 49), or narrow as in *A. nuragica* from Cuccuru Tuvullau, Sardinia (Figs. 47, 50). The different kinds of lunules may contribute to separate species within *Amphiope* if the analysis is based on objective tools, that is the Shape Index (SI) and the Width Index (WI). For this purpose, it was necessary to take data from as many specimens as possible, as suggested by Lambert (1912a; 1915a). Also Cottreau (1914) underlined the necessity of examining lunules in large samples and published the illustration of 15 specimens from Saint Christol (actually “Chemin des Tuilières”), to show the variability in the population from that area.

Another peculiar feature, which can help to characterize a species, is the occurrence of a protruding edge on the aboral face, as in *A. palpebrata* (Pomel, 1885: pl. 11, fig. 4).

The values of SI in some species of *Amphiope* are compared graphically in figure 51. Although in some cases the variability range is wide, the modes are well distinct. The dotted line marks the SI=1.6 value, a level proposed by Stara et al. (2015) to separate the two informal groups *A. bioculata* and *A. nuragica*. The data reported in the graphic were taken from 15 specimens of *A. bioculata* from



Figures 55–60. Oral and aboral plating schemes of two species belonging to *Paraamphiope* from the Rhône Basin (France) and two belonging to *Sculpsitechinus*. Figs. 55, 56: *Sculpsitechinus boulei* (MNHN-F R62136), Aquitanian of Carry-le-Rouet. Fig. 57: *Sculpsitechinus tenuissimus* (neotype, MAC.IVM207), Recent, Lembeh, North Sulawesi (Indonesia). Figs. 58, 59: *Paraamphiope baquiei* (MNHN-F A22689-L18452), Tortonian of Cucuron. Fig. 60: *Paraamphiope agassizi* (MNHN-F.A22687.L18.450-sn2), Rupelian of Pellegrue.

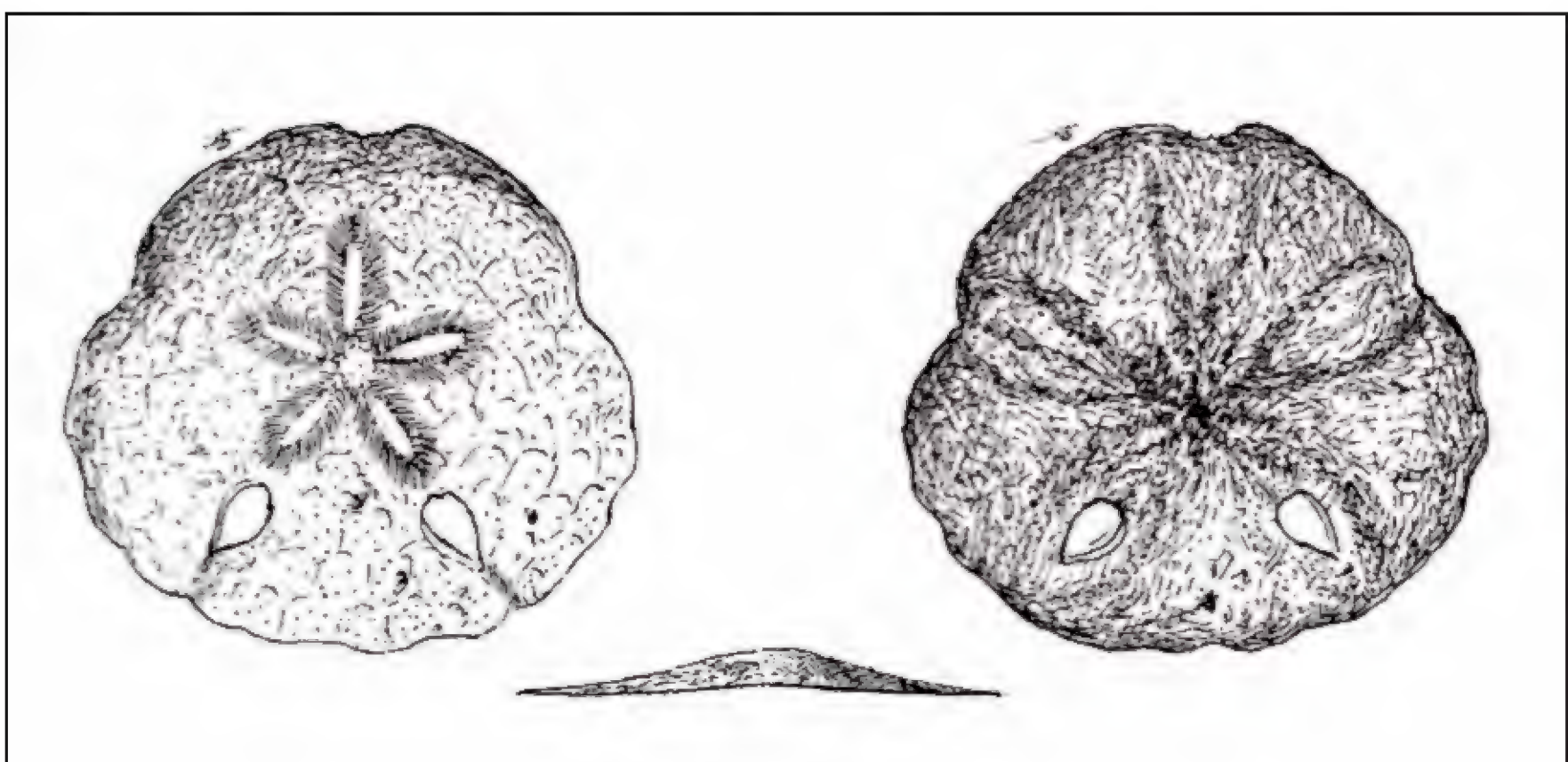


Figure 61. Original illustration of *Paraamphiope baquiei* from Cucuron, with drop-shaped and radially elongate lunules.

Nissan les Tuilières and Lespignan, 12 of *A. romani* n. sp. from Channay sur-Lathan; 10 of *A. montezemoloi* from S. Giorgio and Bonnanaro (Sardinia), 13 of *A. ovalifora* from Gornac, 40 of *A. lovisatoi* from Chiaramonti (Sardinia), 10 of *A. deyderi* from the type locality, 20 whole specimens and more than 100 fragments with complete lunules of *A. nuragica*, from Cuccuru Tuvullao (Sardinia). The graphic (Fig. 51) indicates that SI in *A. bioculata* has a low variability range, similar to that in *A. romani* and *A. montezemoloi*.

The box plot of WI, based on the data taken from eight species (Fig. 53), indicates relevant differences. *Amphiope montezemoloi* is distinctly separated from the other species of the *A. bioculata* group, by the wide variability range of WI (200 to almost 400). *Amphiope romani* n. sp. is not well differentiated from *A. bioculata* also based on WI; however, it differs by other characters, e.g. a much lower value of the distance of the periproct to the posterior test margin (L11; Fig. 52).

Amphiope bioculata, *A. ovalifora* and *A. lovisatoi* are closely related species; also the lunules are rather similar on the basis of the mean values of SI and WI.

However, a comparison between these species based on the test height (TH; Fig. 54) indicates that the mean value of TH in *A. bioculata* is higher than that in *A. ovalifora* and, above all, than that in *A. lovisatoi*. This highlights that also the biometric measurements of the test are necessary for a complete and reliable comparison between populations of *Amphiope* from different localities and/or stratigraphical levels.

In the *A. nuragica* group there is a clear difference in the values of WI between *A. sarasini* and *A. nuragica*.

Petalodium. Also the size of the petalodium has a relevant importance in the taxonomy of *Amphiope*. Although in some species it has a large intraspecific variability, such as in *A. romani* n. sp., its range is commonly rather narrow and can be used as a distinctive character at the specific (Fig. 62) or even generic (Fig. 63) level (see also Stara & Sanciù, 2014; Stara et al., 2015), above all when it is associated to other significant characters.

This hypothesis was confirmed by Stara & Fois (2014), on the basis of a sample made of 30 speci-

mens of Recent *S. auritus* from Tulear (Madagascar). A test carried on a sample of *A. ovalifora* from Gornac (Fig. 64) led to the same results, though it was less clear, likely because the sample was smaller (13 specimens) and the range of the test length (58-90 mm) was smaller than that in *S. auritus*. On the other hand, a similar analysis carried on a sample consisting of 30 specimens of *A. lovisatoi* from Sardinia pointed to a different situation (Fig. 65). Notwithstanding that the value of TL almost doubles in the graphic, the size of PL does not significantly increase in proportion to TL: the mean value of PL in proportion to TL remains almost constant during growth.

This indicates the need of carrying similar tests in all species, when significant samples are available to study.

Food grooves. Food grooves are visible only in well preserved specimens. Sometimes it was possible to take only a part of the pathways, consequently some of the schemes reported in figures 70–75 are composite, that is, taken from a group of specimens to provide the typical situation in each species.

In *Amphiope* the main grooves are finer (Figs. 70–73) than those present in *Paraamphiope* and secondary grooves branch off only distally (with the only exception of *A. sarasini* - Fig. 75). In *Paraamphiope* (Fig. 74) and less distinctly also in *A. sarasini*) fine and short secondary grooves branch off also along the median part of the main grooves, and the two main bifurcations are much more strongly branched distally. Intraspecific variability is present in *Amphiope*: the food grooves system develops by an increasing degree of secondary branchings from *A. bioculata*, to *A. ovalifora*, *A. lovisatoi* and finally *A. sarasini*. *Amphiope nuragica* (Fig. 71) shows the simplest scheme so far known in *Amphiope*, since the distal secondary grooves are, on the whole, less numerous and shorter than in *A. bioculata*.

On the whole, the present study highlighted the necessity of analysing all these characters when comparing populations from different localities, since apparently similar forms can be separated at the specific, sometimes even at the generic level, if one or more of these features turn out to differ significantly.

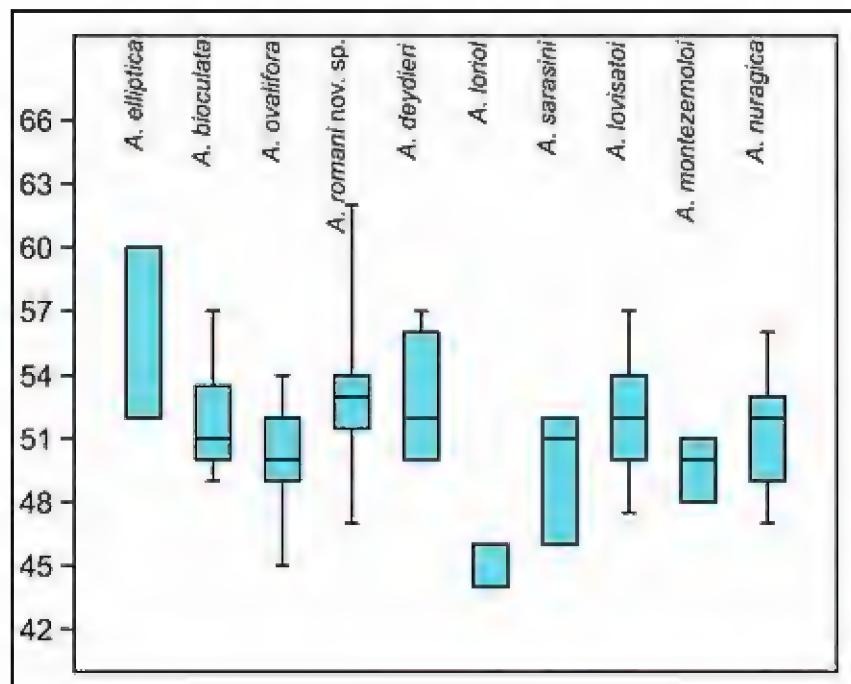


Figure 62. Box-plot showing the mean values and the variability ranges of PL (size of the petalodium) in ten species of *Amphiope*.

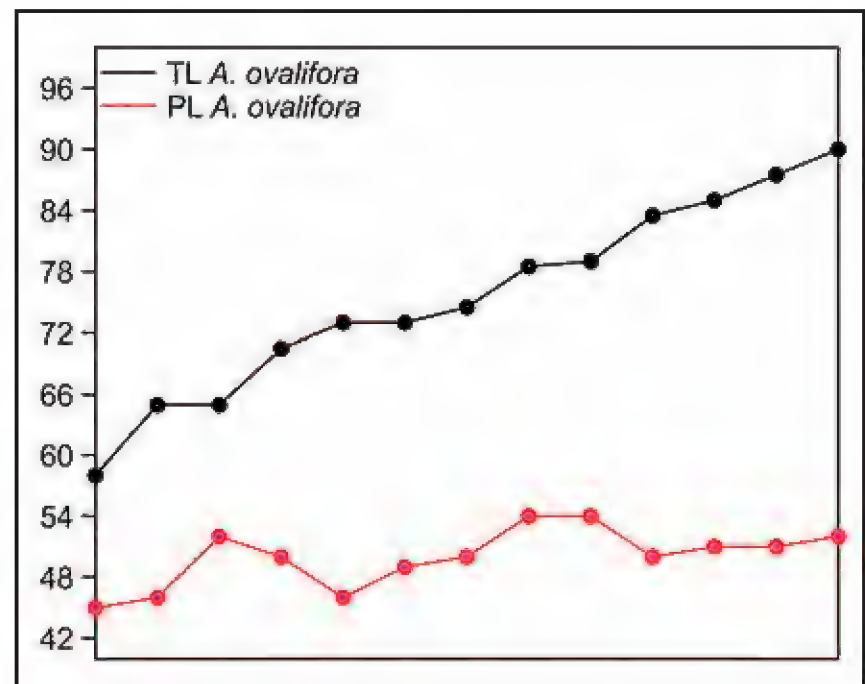


Figure 64. *Amphiope ovalifera*, topotypic sample from Gornac: graphic showing the variation of PL (size of the petalodium) during growth.

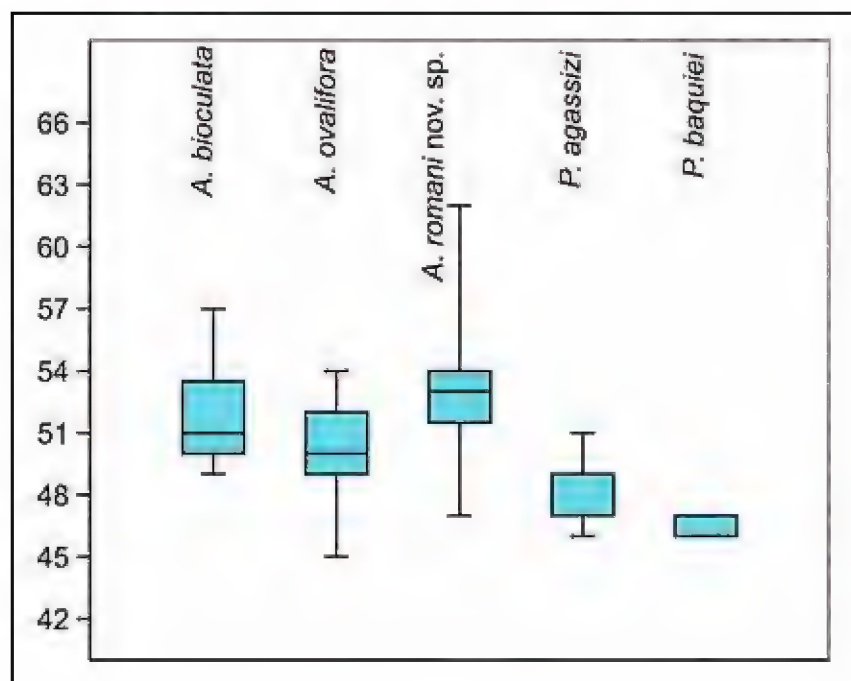


Figure 63. Box-plot comparing the mean values and the variability ranges of PL (size of the petalodium) in three species of *Amphiope* and two of *Paraamphiope*.

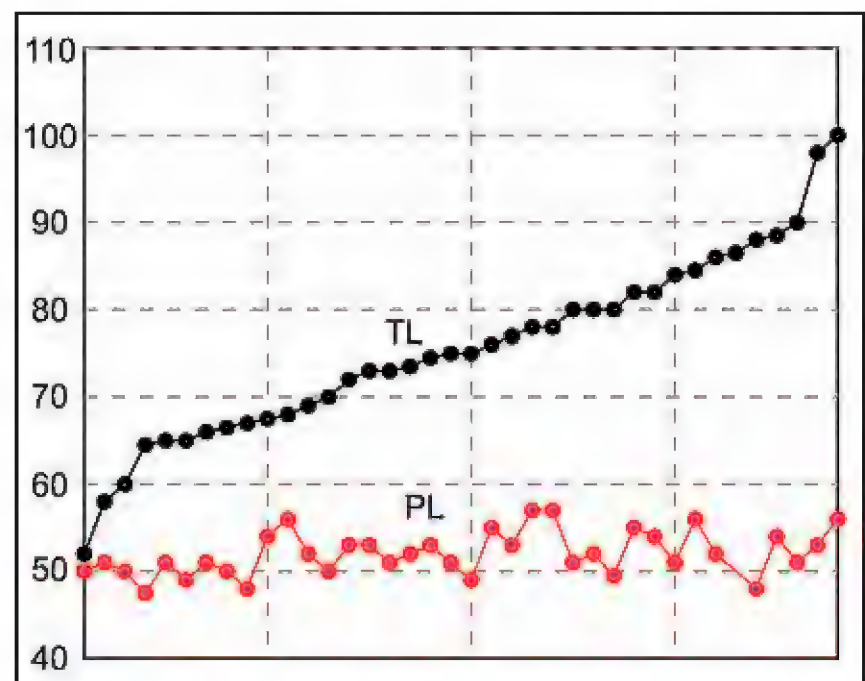
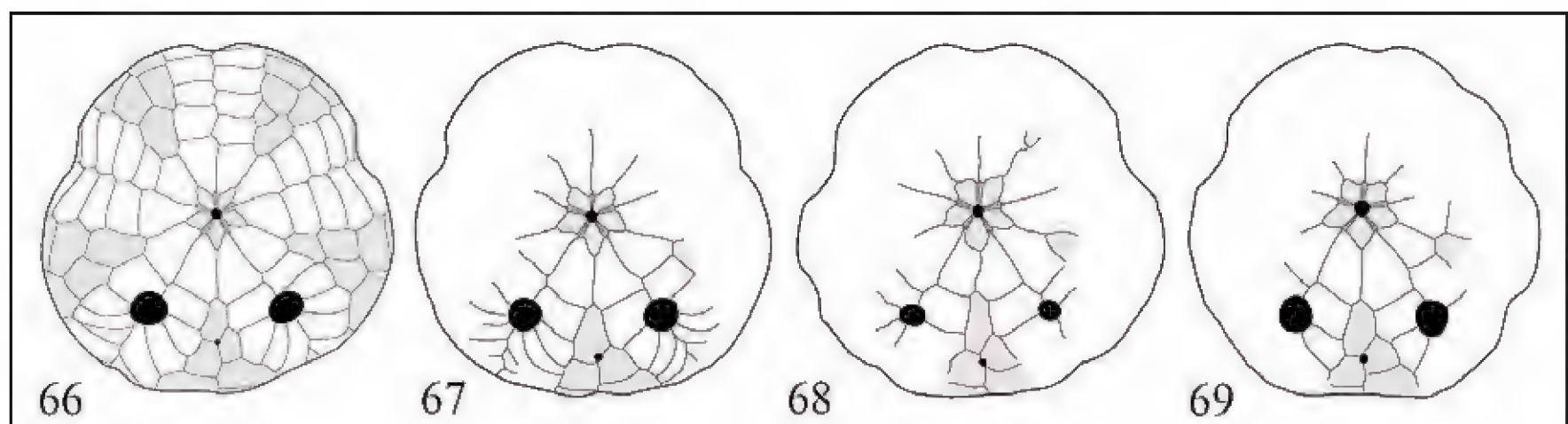


Figure 65. *Amphiope lovisatoi*, topotypic sample from Chiaramonti (Sardinia): graphic showing the variation of PL (size of the petalodium) during growth.



Figures 66–69. *Amphiope bioculata*, topotypic specimens from Lespignan-Nissan (MNHN-F): oral plating schemes showing the variability in the plate shape and arrangement in the interambulacrum 5 and in the position of the periproct. Fig. 66: specimen which will be proposed as neotype (A 57777). Fig. 67: specimen A 57778. Fig. 68: A.22701.L18464f. Fig. 69: A. 22701.L18464h.

Species of Amphiope represented by scanty material

A part of the species examined are represented by scanty or badly preserved material. They are briefly discussed in the following. Only a few of them are here accepted as valid species.

Amphiope perspicillata Agassiz, 1841 was based on a sole badly preserved specimen from “terrains tertiaire” of Rennes (northwestern France), without the indication of the exact finding locality. A doubtful plating scheme was reported in the original illustration. The specimen is wanting and also Lambert (1907, 1912a) was not able to trace it. For these reasons *A. perspicillata* is here considered as *species inquirenda*.

Amphiope styriaca Hoernes, 1883, from Seggaubert, Styria (Austria). A plating scheme was provided by Kroh (2005), who synonymised it with *A. bioculata*. However, the sole known specimen is incomplete and lacks a large part of the posterior margin in the interambulacrum 5, thus preventing reliable comparison with the other known species. Therefore, *A. styriaca* is considered as *nomen dubium*.

Amphiope palpebrata Pomel, 1887. Only one out of three specimens from the Cartennian (Burdigalian?) of Djebel Djambeïda (Cherchell, Algeria) was figured by Pomel (1887: pl. 11, figs. 1–4). However, Pomel admitted that the illustration did not correspond to the real preservation of that specimen. No plating scheme can be taken from the original illustration. The lunules are different from those present in the other species which were known at that time. A specimen (MNHN-F. L18.478) collected by Cotteau et al. (1891) from the type-locality, provided us with a partial plating scheme (Figs. 155, 156) and a radiography (Fig. 87), which are different from those in the other species known at that time. For this reason, we temporarily maintain *A. palpebrata* as valid. Studies are in progress to revise the Algerian species of *Amphiope* (personal communication, Mohamed Belkercha, June 2016).

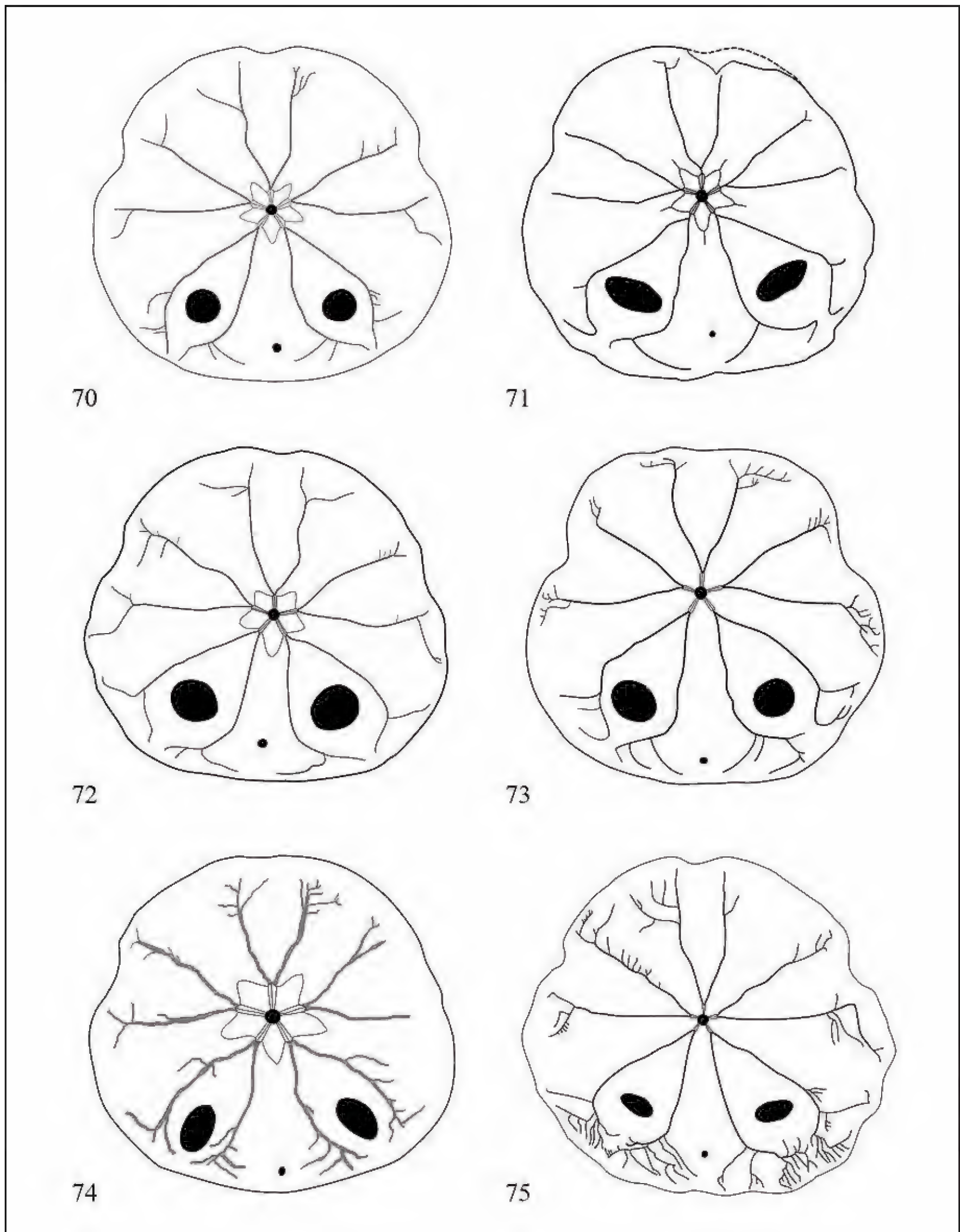
Amphiope depressa Pomel, 1887. The illustration (Pomel, 1887: pl. XII, figs. 1a-c) of the sole specimen known from the “Helvetian” (Langhian? Serravallian) of Aïn-el-Arba (Mléta, Orano, Algeria) was incomplete since a part of the posterior margin was lacking. It is not possible to take a

plating scheme from that illustration. The specimen is very close to *A. sarasini* from the Tortonian of the Rhône Basin, however we maintain here *A. depressa* as valid and separated from *A. sarasini*, since the lunules have a peculiar shape and the values SI and WI are almost the lowest in the genus *Amphiope*. Furthermore, according to Pomel (1887), *A. depressa* has 5 genital pores; if this character will be confirmed by new findings, the systematic of this taxon should be revised. Another research group is trying to find out new material to revise this species (personal communication, Mohamed Belkercha, June 2016).

Amphiope villei Pomel, 1887 and *A. personata* Pomel, 1887 were not originally illustrated and the descriptions did not allow a reliable comparison with the other known species of *Amphiope*. Also the repository of the type-material was not indicated. A part of the Pomel collection was bought by a private collector who finally gave it to the Metropolitan Museum of New York (Cleevely, 1986). Another part was obtained by the NHMUK (Woodward, 1904); however, no specimens of *Amphiope* are present among those specimens (personal communication, Bajo Campos, February 2016). Therefore, the type material is wanting. Pomel (1887) indicated the respective type localities in the original descriptions, however Cotteau et al. (1891) were not able to find out any specimen from there, and it was not possible to trace the localities since the old toponyms are actually unknown. For these reasons these two species are here considered as *species inquirenda*.

Amphiope neuparthi de Lorient, 1905, from the Burdigalian of Luanda (Angola). The X-ray photo provided by Darteville (1953) of this species shows a peculiar internal structure when compared with the other species of the *A. bioculata* group, in particular with *A. montezemoloi* which shares large roundish lunules. Additionally, a characteristic notch on the posterior margin of the test is present in the original illustration. For these reasons *A. neuparthi* is here maintained as a valid species. A research is in progress based on the type material of this species (personal communication Pedro Pereira, July 2016), which was not available to the present study.

Amphiope dessii Cotteau, 1895 and *A. calvii* Lovisato, 1914. The type localities, represented by ancient toponyms, have been traced on old maps, but



Figures 70–75. Schemes of the food grooves in species of *Amphiope* and *Paraamphiope*. Fig. 70: *A. bioculata* (MNHN- F.A 57777), Langhian-Serravallian of Lespignan. Fig. 71: *A. nuragica* (MAC PL1590). Cattian-Aquitania of Cuccuru Tuvullau, Sardinia. Fig. 72: *A. ovalifera* (MNHN-F A 22710-L 18.447f), Aquitanian (Burdigalian?) of Gornac. Fig. 73: *A. lovisatoi* (MAC.PL2014), late Burdigalian of Chiaramonti (Sardinia). Fig. 74: *P. agassizi*, Rupelian of Pellegrue. Fig. 75: *A. sarasini* MNHN-F.A.57788 , Tortonian of Cruzy.

the original outcrops disappeared due to the enlargement of the village of Nurri (Cagliari Province), in the case of *A. dessii*, and of the village of Ploaghe (Sassari Province), in the case of *A. calvii*. The original description and illustration do not provide characters for a reliable comparison and point to forms close to *A. nuragica* and *A. lovisatoi*, respectively. For these reasons *A. dessii* and *A. calvii* are here considered as *species inquirendae*.

Amphiope bioculata philodonax was proposed by Lambert (1927: p. 112) for the specimen MHNbX 2014.6.189, which was labeled by des Moulins as “type of *Scutella bioculata* var. *A. from Saucats, Gironde, France*”. This specimen is heavily encrusted and does not provide sufficient data for a reliable classification. For these reasons *A. bioculata philodonax* is here considered as *subspecies inquirenda*.

Another variety, *A. bioculata* var. *aequipetala* Lambert, 1927 (Lambert, 1927: 113), was based on a single specimen with rounded lunules from “Gers”. In this case the repository of the type material was not provided and the stratigraphic position is uncertain. Since the characters described to separate them were unclear, *aequipetala* is here considered as *subspecies inquirenda*.

Amphiope bioculata var. *drunensis* Lambert, 1915. Only two specimens (syntypes: MNHN-F. A22379-L18.468 and 18.457; Figs. 7, 8) from the Langhian of St.-Paul-Trois-Châteaux (Drôme) were available to study and field research at the same locality by one of us (PS) did not provide new material. Those specimens could be easily included within the morphological variability range of the type-series of *A. bioculata* from Hérault, with the exception of the lunules, which are much more ovalised. On the basis of this last feature this variety is here maintained as valid subspecies.

A group of four species earlier attributed to *Amphiope* were described from the Miocene of Libya and Egypt: *A. truncata* Fuchs, 1882, *A. arcuata* Fuchs, 1882, both from Oasis Siouah (Egypt), *A. fuchsi* Fourtau, 1901 and *A. miocenica* Ali, 1998, by Fourtau (1899; 1900; 1920); Gregory (1898; 1911); Ali (1998; 2014). No plating schemes and other distinctive characters, necessary for a reliable attribution, were provided in the original descriptions.

Fourtau (1901) renamed *A. truncata* as *A. fuchsi*, affirming that the denomination *A. truncata* given by Fuchs in 1882 was pre-occupied by *Lobophora*

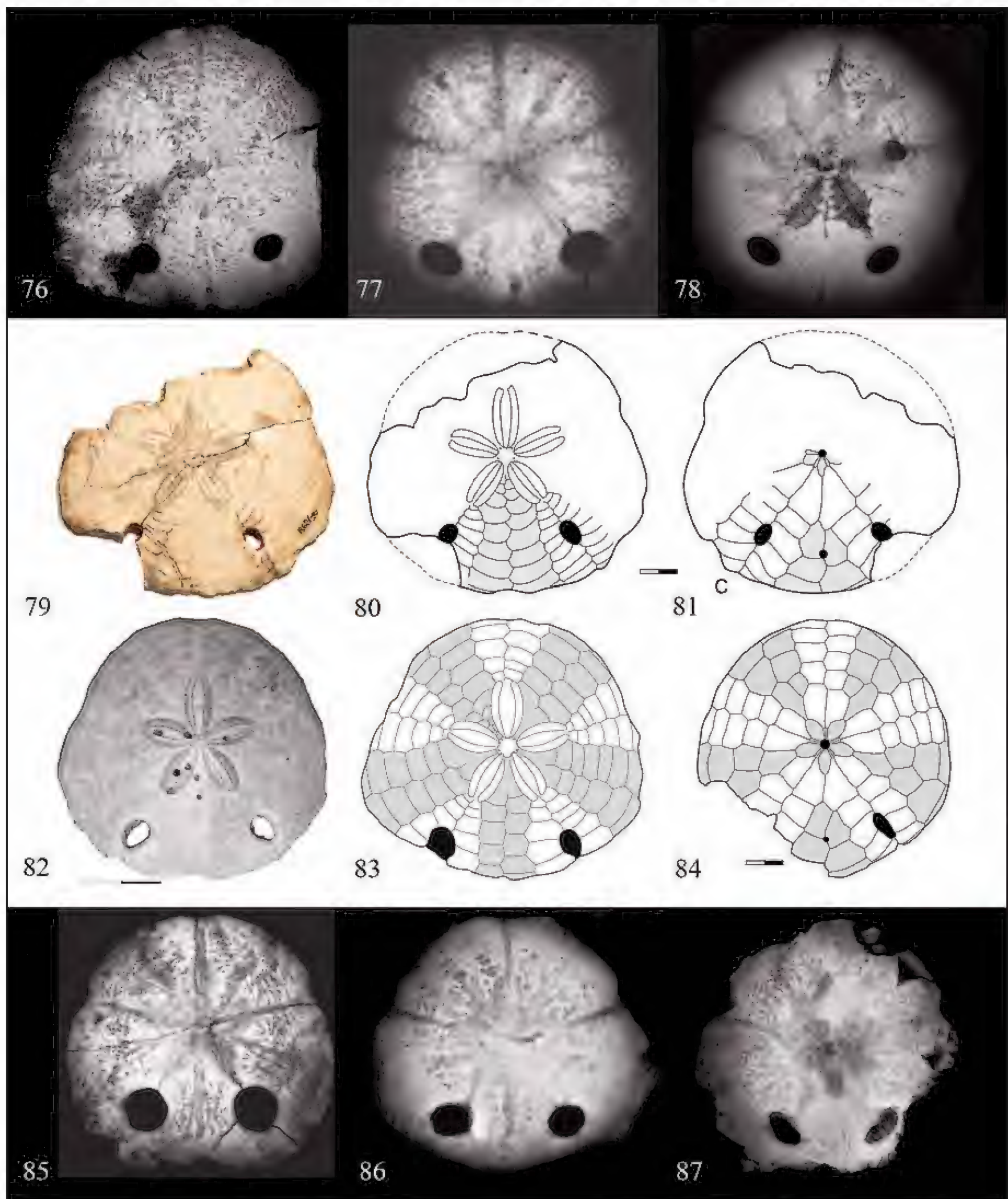
truncata L. Agassiz, 1841, and that *Amphiope* and *Lobophora* have been subsequently reunited by Agassiz & Desor (1847). However, *L. truncata* was maintained into the genus *Lobophora* by Agassiz & Desor (1847: 78); additionally, it was represented by a very different specimen, which subsequently turned out (Forteau, 1901) to belong to the genus *Echinodiscus* as claimed by A. Agassiz (1872-74) in his revision of the genus. Consequently *A. fuchsi* is a junior synonym of *A. truncata*. Indeed, Cottreau (1914) considered *A. arcuata* and *A. fuchsi*, from the Middle Miocene of Egypt, as junior synonyms of *A. truncata*.

The type specimen of *Amphiope miocenica* Ali, 1998, from the Langhian-Serravallian of Siwa (Egypt), is very similar to the two specimens of *Paraamphiope arcuata* (NHMUK E76161-2) in Stara & Sanciú (2014, pl. 20). However, it has a larger petalodium (PL= 52% TL, against 42–47% TL in *P. arcuata* and in *A. truncata*), a larger space separates the petal tips from the corresponding lunules and the food grooves were described as strongly branched by Ali (1998). For these reasons that specimen probably does not belong to the genus *Amphiope*. Recently, Ali (2014) compared the species *miocenica*, *fuchsi* and *arcuata* using a consistent morphometric data set; however, he did not provide plating schemes for them.

The taxonomic position of *Amphiope arcuata* has been recently discussed by Stara & Sanciú (2014), who transferred it to the genus *Paraamphiope* based on the plating schemes of five specimens examined at the NHMUK (E76161-2, E76164-6). The illustration provided by Fuchs of the type of this species (1882: pl. 11, figs. 4-6) corresponds to the specimens examined at the NHMUK.

On the basis of the data published so far, the species *truncata* and *miocenica* are here considered as separate species. However, since no plating schemes were provided in the original descriptions, their generic placement remains uncertain, although the attribution to the genus *Paraamphiope* looks probable.

The scheme of the oral plate arrangement is needed for the generic attribution when dealing with species belonging to the genera *Paraamphiope* and *Sculpsitechinus* (see also Stara & Sanciú, 2014), since the sole aboral plate arrangement is not sufficient for a reliable diagnosis.



Figures 76–78. X-ray photographs of three species of *Amphiope*. Fig. 76: *A. elliptica* (MNHN-F A22706-L18.471), from Carry. Fig. 77: *A. bioculata* (MNHN-F A 57778), Lespignan. Fig. 78: *A. ovalifera* (MNHN-F A 22710-L 18.447m), Gornac. Figures 79–84. *Sculpsitechinus boulei* (MNHN-F R62136). Fig. 79: aboral view. Figs. 80–81: aboral and adoral plating schemes, respectively. *Paraamphiope arcuata*: Fig. 82: aboral view, Fig. 83: aboral plating scheme of the specimens NHMUK.E76162 and NHMUK.E76164, respectively. The presence of at least three plates or more between the petal tips and the respective lunules excludes the attribution of these specimens to the genus *Amphiope*, however this character alone cannot safely distinguish between *Sculpsitechinus* and *Paraamphiope*. Fig. 84: adoral view of *S. tenuissimus* (neotype, MAC.IVM207), Recent, Lembah, North Sulawesi, Indonesia) to compare the adoral view of *S. boulei*. Figures 85–87. X-ray photographs. Fig. 85: *A. montezemoloi* (MACPL1677), Ardara (Sardinia). Fig. 86: *A. lorioli* (MNHN-F A22707-L 18472Aa). Fig. 87: *A. palpebrata* (MNHN-F. L18.478), Djebel Djambeida (Cherchell, Algeria).

Amphiope laubei Lambert, 1912. Lambert (1912) affirmed that the specimen from Austria attributed by Laube (1871) to *A. elliptica* did not belong to that species and instituted for that specimen a new species, *A. laubei*. After Cottreau (1914) that specimen was deformed and the periproct was not visible, and was likely attributable to *A. bioculata* from Hérault. Kroh (2005), when studying the specimen from Niederkreuzstetten (NHMW 11849/0023/0039) figured in Laube (1871: pl. 16, fig. 5), suggested that the original illustration was likely a composition of a number of different specimens and synonymised it with *A. bioculata*. For these reasons and the bad preservation of the type material, *A. laubei* is here considered as *nomen dubium*.

Amphiope doderleini (Lambert et Thiéry, 1921). After Kroh (2015), it is a junior synonym of *Echinodiscus truncatus* Agassiz (1841), by incorrect original spelling (ICZN 4th ed. Art. 32.5.1., inadvertent error). See also Stara & Sanciù (2014).

Amphiope labriei Lambert, 1927. This species was based on a sole specimen with the posterior edge damaged; the aboral plating scheme (Fig. 24) was subsequently provided by Lambert (1928). The finding locality was not visited by Lambert, who admitted that the stratigraphic position of that specimen was unknown and only tentatively was attributed to the “Helvetian”. That specimen was considered by Lambert (1928) as similar to *A. baquiei*, by the lunule shape and the plating arrangement

around them. No specimens attributed to this species have been found in French Institutions. For these reasons *A. labriei* is here considered as *species inquirenda*.

Amphiope dallonii Lambert, 1931. Lambert (1931) received a specimen of *Amphiope* from the Helvetian of Thouanet (Algeria) by M. Dalloni. Given that the specimen was very deformed and with surfaces indented by sandstone clasts, he admitted that it was possible to provide only an incomplete diagnosis (in our opinion, not enough to create a species). Since no repository indication has been reported, we consider this species as *species inquirenda*.

Amphiope bioculata var. *pelatensis* Fabre, 1933, was based on a number of middle-sized specimens with rounded lunules from Pelat, an unknown locality in the Municipality of Condom (Gers). No illustration and repository indication were provided. The sample shows a large morphological variability and the biometric data provided in the original description do not allow a reliable discussion. A nearby locality (Montréal, Gers), was indicated by Lambert (1915) as the type-area for *A. bioculata turonensis*.

Amphiope bioculata var. *bentivegnae* Desio, 1934 was described from the Middle Miocene of Libya. No illustration and indication about the repository of the type material was provided for both these subspecies. These three subspecies are here considered as *subspecies inquirendae*.

Valid species included in the genus *Amphiope*

As a result of this research 17 valid species and a subspecies are here assigned to the genus *Amphiope* (see the list in the systematic chapter), including also those discussed in Stara & Borghi (2014).

A new species, *A. romani* nov. sp., is here instituted on the basis of a group of specimens from the Serravallian-Tortonian of Channay-sur-Lathan, Touraine (France), since the combination of its morphological features distinguishes it from the other known taxa.

Species of *Amphiope* transferred to other genera

Three species earlier assigned to *Amphiope* have been transferred to the genus *Paraamphiope* Stara

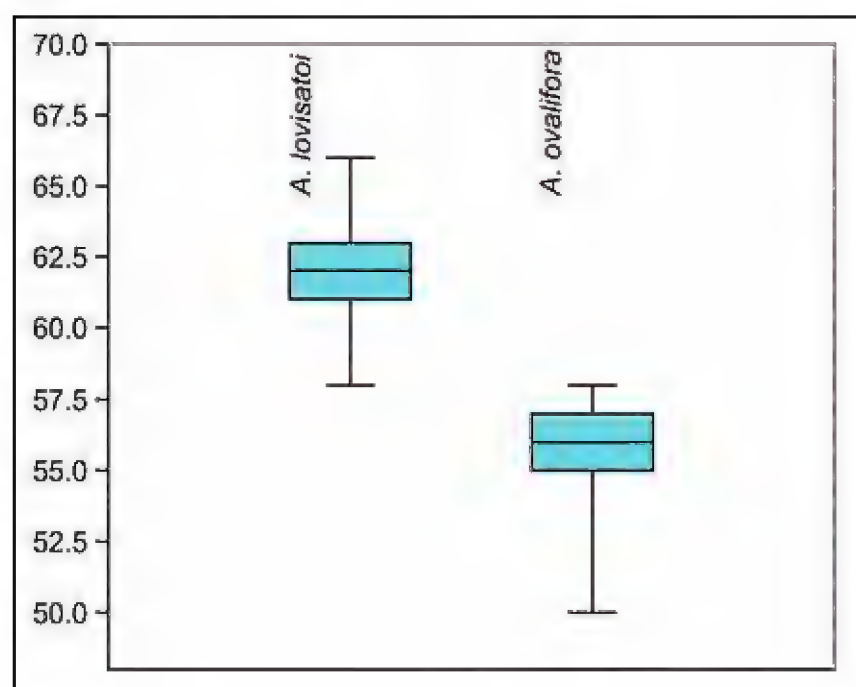


Figure 88. Box-plot comparing the mean values and the variability ranges of L4 (position of the apical disc), expressed as % TL, in *A. lovisatoi* (N30) and *A. ovalifera* (N15).

& Sanciú, 2014, since they have radially elongate lunules separated by at least three-four plates from the posterior petals tips, small or rather small petalodium, plates 5.b.2 and 5.a.2 paired or almost paired and strongly branched oral food grooves:

P. agassizi (Des Moulins in Cotteau, 1864)

P. cherichirensis (Thomas & Gauthier in Gauthier, 1889)

P. baquiei (Lambert, 1907).

Also *A. arcuata* Fuchs, 1882 was placed into the genus *Paraamphiope*, by Stara & Sanciú (2014). Another species earlier attributed to *Amphiope*, with very branched food grooves and axial lunules separated from the posterior petals by four plates and with the plating that matches that of *Sculpsitechinus tenuissimus*, has been assigned to the genus *Sculpsitechinus* Stara et Sanciú, 2014: *S. boulei* (Cotteau, 1914).

Intermediate cases between *Amphiope* and the closely related genera

The distinctive characters separating *Amphiope*, *Paraamphiope*, *Sculpsitechinus* and *Echinodiscus* were stated in Stara & Sanciú (2014). However, some intermediate cases have been encountered in this study and are described in the following.

All the species with radially elongate lunules examined in this study and in Stara & Sanciú (2014) belong to the genera *Paraamphiope*, *Sculpsitech-*

inus or *Echinodiscus*. However this kind of lunule may be rarely present also in *Amphiope* (Figs. 46, 49). They must be considered as border cases within the variability range of a population with mainly roundish or transversely elongate lunules. The occasional occurrence of radial elongate lunules was observed also by Philippe (1998a) when studying the populations of *Amphiope* from the Rhône Basin; unluckily, it was not possible for us to visit the Museum des Confluences of Lyon, where those specimens are stored.

As underlined above, the plate structure of the test is one of the most useful tools for a reliable classification at the generic level. However, also in this case exceptions have been observed. In some specimens of *A. romani* n. sp. from Touraine (Figs. 161, 163) the structure of the interambulacrum 5 and the number of plates separating the petals from the respective lunules are similar to those in *Paraamphiope raimondii* (Fig. 10), and the oral interambulacral plate 5.b.2 is sometimes wide and short, thus resembling the typical shape in *Paraamphiope*, although the structure of the plates around the lunules and the interior floor of the central hollow are typical of all other *Amphiope* species.

Also in a few specimens of *A. sarasini* (e.g. the syntype under study, Fig. 43) there are three plates between the petal tips and the corresponding lunules and the food grooves are strongly branched, thus resembling those in *Paraamphiope*; however they

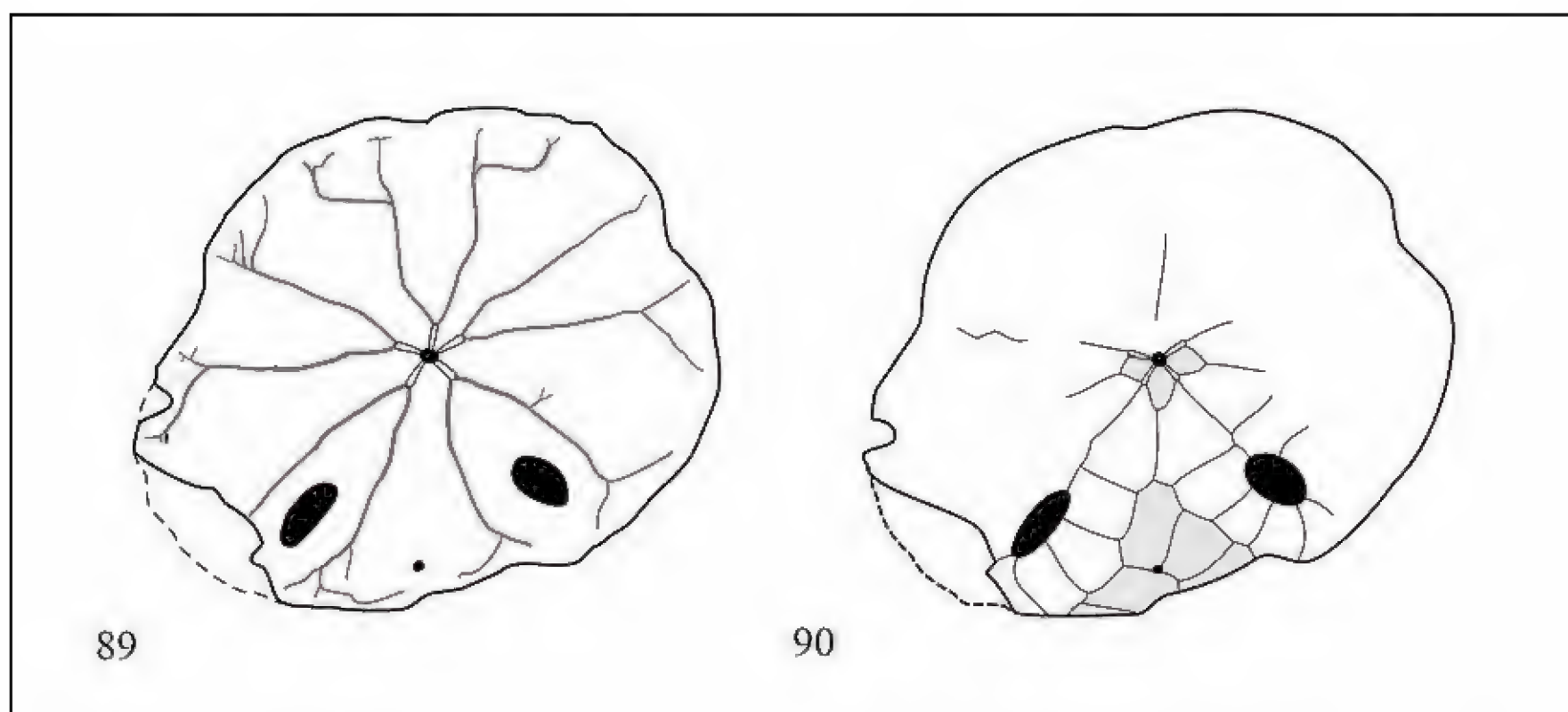


Figure 89, 90. *Paraamphiope cherichirensis*: food grooves and adoral plating in MNHN-F.R67287, Bou Golrine (Libya).

have the interambulacral plate 5.b.2 much staggered with respect to 5.a.2 and clearly transversely elongate lunules, which are typical characters of *Amphiope*.

In the studied specimen of *Sculpsitechinus boulei* (Aquitanian) the plating scheme of the oral interambulacrum 5 (Fig. 56) corresponds to that present in the Recent *S. tenuissimus* from Lembeh (Indonesia; Fig. 57, Fig. 84); this also applies to the number of plates separating the petal tips from lunules (Stara & Sanciù, 2014: fig. 21A and pl. 22, fig. 3). Instead, in some specimens of the Recent *Sculpsitechinus* sp. 1 from the Philippines (Stara & Sanciù, 2014: pl. 11, fig. 3) and from Iran (Fatemi et al., 2016: pl. 2 B, D) only the oral interambulacral plate 5.b.2 is in contact with the ambulacral plates I.a.2 and V.b.2, as in *Amphiope* and *Paraamphiope*. On the other hand, this species has always a high number of plates in the oral interambulacrum 5, ranging from three to four plates in column “a” and three to five in column “b”, as in the typical *Sculpsitechinus*.

All these intermediate cases underline the need of analysing all the features present in a population, since a sole distinctive character could not provide alone a safe generic attribution if not supported by other significant features.

SYSTEMATICS

Family *Astriclypeidae* Stefanini, 1912

Genera included: *Echinodiscus* Leske, 1778; *Astriclypeus* Verrill, 1867; *Amphiope* L. Agassiz, 1840; *Paraamphiope* Stara et Sanciù, 2014; *Sculpsitechinus* Stara et Sanciù, 2014

Genus *Amphiope* L. Agassiz, 1840

TYPE SPECIES. *Scutella bioculata* Des Moulins, 1837, by subsequent designation of Lambert (1907: 49).

EMENDED DIAGNOSIS. Partially modified from Smith & Kroh (2011) and Stara & Sanciù (2014). Test low with the highest point corresponding to its centre or slightly anterior of it. Margin thin. Oral side flat to slightly concave. Internal test support well developed, consisting of pillars and walls

crossed by cavities; one of them extends along the interambulacrum 5 and terminates into the periproct; towards the ambitus, sometime the peripheral ballast system becomes denser, even massive, and crossed by microcanals. Apical disc monobasal, subcentral or slightly anterior of centre, with four small gonopores. Ambulacra and interambulacra similar in size at ambitus. Petals well developed; short (about half radial length of test), almost closed distally. All five petals similar in length; sometimes the frontal petal slightly longer than the others. Petalodium small to middle sized (40,5–62% TL; see Stara et al., 2015), with β angle about 88°. Oral interambulacra narrower than the ambulacra, even at their widest point. Oral interambulacra 1, 4 and 5 always with only the first post-basicoronal plate in contact with the first pair of ambulacral plates; the interambulacral zones are separated by a couple of enlarged first post-basicoronal ambulacral plates. Oral interambulacra 2 and 3 may have only the plate 5.2.b, or both plates 5.2.b and 5.2.a, in contact with the two adjacent ambulacral postbasicoronals.

Basicoronal circlet pentastellate with interambulacral plates forming the points. Two to five post-basicoronal plates per column present in the interambulacrum 5 adorally. Two rounded or transversely elongate lunules are present in the posterior ambulacra; they are separated from the tips of the corresponding petals by only one-two (rarely three) couples of plates. Peristome small, subcentral or slightly anteriorly located. Periproct circular, small, opening between the first, the second or the third pair of post-basicoronal interambulacral plates. Food grooves are rather thin and develop by a simple scheme; the main trunk bifurcates a short after the edge of the basicoronal plate into two main grooves which extend towards the test margin, but not reaching it; distal branches present, whereas almost no secondary grooves branch off the middle part of the main grooves. Posterior pair of the food grooves running around the lunules and behind the periproct. Tuberculation dense, made of very small, perforate and crenulate tubercles. Tubercles are larger on the oral face, with the exception of those located along the food grooves.

DISTRIBUTION. From Oligo-Miocene to Pliocene. Central and Southern Europe, North Africa, Middle East, India, Angola (Smith & Kroh, 2011).

Species and subspecies included:

- Amphiope bioculata* (Des Moulins, 1837), Langhian-Serravallian, Hérault (France)
A. bioculata var. *drunensis* Lambert, 1915, Langhian, Drôme (France)
A. depressa Pomel, 1887, Miocene, Algeria
A. deydieri Lambert, 1912, Langhian-Serravallian, Vaucluse (France)
A. elliptica Desor, 1847, late Aquitanian, Bouche du Rhône (France)
A. hollandei Cotteau, 1877, Burdigalian, Corsica (France)
A. lorioli Lambert, 1907, Tortonian, Hérault (France)
A. lovisatoi Cotteau, 1895, late Burdigalian, Sardinia (Italy)
A. ludovici Lambert, 1912, Tortonian, Vaucluse (France)
A. montezemoloi Lovisato, 1911, late Burdigalian-early Langhian, Sardinia (Italy)
A. neuparthi de Loriol, 1905, Miocene, Luanda (Angola)
A. nuragica (Comaschi Caria, 1955), late Chattian-early Aquitanian, Sardinia (Italy)
A. ovalifora Des Moulins in Fallot, 1903, late Aquitanian, Gironde (France)
A. pallavicinoi Lovisato, 1914, late Burdigalian, Sardinia (Italy)
A. palpebrata Pomel, 1887, Miocene, Algeria
A. romani n. sp., Serravallian-Tortonian, Touraine (France)
A. romani turonensis (Lambert, 1915), Serravallian-Tortonian, Touraine (France).
A. sarasini Lambert, 1907, Serravallian -Tortonian, Hérault (France)
A. tipasensis (Aymé & Roman, 1954), Pliocene, Algeria
A. transversifora Lambert, 1910, Langhian, Drôme (France)

Amphiope bioculata group (sensu Stara et al., 2015)

This informal group includes species with rounded or transverse elliptical lunules, with a maximum SI <1.6: *A. bioculata*, *A. elliptica*, *A. lovisatoi*, *A. ovalifora*, *A. neuparthi*, *A. lorioli*, *A. montezemoloi*, *A. ludovici* and *A. romani* n. sp. Remarks about *A. neuparthi* are reported in the discussion chapter, for *A. lovisatoi* and *A. montezemoloi*, see Stara & Borghi (2014).

Amphiope bioculata (Des Moulins, 1837) Figs. 27, 28; Figs. 91–99; Figs. 66–69; Figs. 7, 8; Fig. 9; Fig. 70; Fig. 77

1791. *Scutella bifora* Lamarck - Bruguières: pl. 147, figs. 5-6
 1816. *Scutella bifora* Var. 3 Lamarck: p. 10
 1837. *S.[cutella] bioculata* Nob. Des Moulins: p. 226
 1840. *Amphiope bioculata* (Desmoulins) - Agassiz: p. 6
 Non 1841. *Amphiope bioculata* (Desmoulins) - Agassiz: p. 73; pl. 11, figs. 1, 5
 1847. *Lobophora bioculata* Agassiz - Agassiz & Desor: p. 78
 1902. *A. bioculata* (Desmoulins) - de Loriol: p. 21; pl. 2, figs. 4, 6
 1907. *A. bioculata* Desmoulins (*Scutella*) - Lambert: pp. 50-53
 1912a. *A. bioculata* Desmoulins (*Scutella*) - Lambert: pp. 75-77
 1912a. *A. bioculata* var. *drunensis* Lambert: pp. 77, 85.
 1914. *A. bioculata* Desm. (*Scutella*) - Cottreau: pp. 135-139; pl. 5, figs. 1-8; pl. 6, figs. 1-12.

TYPE LOCALITY. Des Moulins indicated “Sure près Bollène”, an unknown locality in Vaucluse (France). After de Loriol (1902), Lambert (1912a, 1926) and Cottreau (1914) the Langhian-Serravallian of Nissan-Lespignan (Hérault, France) is the type-area for this species.

TYPE MATERIAL. Two syntypes, the specimen MNHBx 2014.6.317 from “Sure” and A-MHNBx 2014.6.189 (2) from Saucats Gironde) were indicated as types of *Scutella bioculata* var. A by des Moulins (1837 and original label). However the latter was attributed to the “variety” *A. bioculata philodonax* by Lambert (1927).

OTHER EXAMINED MATERIAL. 11 whole specimens from the surroundings of Nissan (Hérault), in the type-area; three of them (MNHN-F.A 57777-9) from Lespignan, and 8 (A2270.L18464a-h) from Nissan les Tuileries (Chemin des Tuilières). All from the Langhian - Serravallian. A well preserved specimen (MNHN-F.A57777) from Lespignan will be proposed to the ICZN as neotype.

DESCRIPTION. Middle sized species (mean in our sample TL=61 mm, range 55–74 mm), with sub-

circular outline (mean TW=107% TL) and restricted anteriorly. Rather deep notches in correspondence of the anterior ambulacra. The test is high (mean TH=16.5% TL). Internal structure: central hollow vaguely pentagonal-starry shaped, with the rear wall located between the two posterior paired ambulacra and at the right side of the interambulacrum 5. From the rear wall to the front, the length of the hollow is about 50% TL. Five long and wide macrocanals, starting from the central hollow, extend along the interambulacra. The internal ballast system is very reduced, with large spaces extending towards the test margin. Apical system anteriorly eccentric (mean L4=57% TL), broad (9.5% TL). Petalodium size variable (PL=49-57% TL, mean PL=52% TL). Frontal petal longer than the posteriors (mean L5=25% TL, mean L9=23% TL). In the frontal petal L6=60% L5, in the posteriors L10=70% L9. Interporiferous zones slightly wider than a poriferous one. In the oral interambulacrum 5 the column "b" is made of three post-coronal plates, column "a" of two with sometimes a part of the third plate. On the whole there is the same quantity of plates (13-15) in each column of the interambulacrum 5 and of the ambulacra I and V. The size of the basicoronal circlet is variable (L13=12-16% TL). The mean length of the oral plate 5.b.1 is 8% TL. Lunules variable in size (range WI=56-145), though commonly middle-sized (mean WI=114), rounded to slightly transversely ovoidal (mean SI=1.22, range SI=1-1.52). They are separated by two (seldom one) plates from the tip of the correspondent petals (L3=5.5% TL). Peristome middle-sized (range ϕ ps=3.4-4% TL) and located centrally (mean L12=50% TL). Periproct small (mean ϕ pc=2% TL), rather far from the posterior test margin (mean L11=11.6% TL) and located between plates 5.a.2-5.b.3 or 5.a.2-5.b.2, or at the conjunction of plates 5.b.2-5.a.2-5.b.3. Food grooves developing by a simple scheme, though sometimes well branched distally, and not reaching the margin. No secondary ramifications branch off along the main grooves. A short branch of the posterior grooves extends towards the rear of the periproct, but does not reach it.

DISTRIBUTION. Langhian-Serravallian (after Roman, 1974) of Nissan and Lespignan (Hérault-France); Aspiran (Hérault); St.Paul-Trois-Châteaux (Drome; MNHN-F. A22379.18.457, specimen in figures 7, 8).

Amphiope elliptica Desor, 1847 - Figs. 25, 26; Figs. 100-105; Fig. 76

1847. *Lobophora elliptica* Desor in Agassiz & Desor: p. 78

1858. *Amphiope elliptica* - Desor: p. 236

1907. *Amphiope elliptica* Desor - Lambert: p. 54

1914. *Amphiope elliptica* Desor - Cottreau: p. 94; figs. 19-20; pl. 8, fig. 1.

TYPE LOCALITY. Desor (in Agassiz & Desor, 1847) indicated S.te Restitut (Drome - France) and Carry, près de Martigues. However, Cottreau (1914) affirmed that the true type locality was Carry le Rouet (Bouche du Rhône), late Aquitanian-early Burdigalian (after Roman, 1974).

TYPE MATERIAL. The holotype and its cast (T93) are wanting; they were not cited in the catalogue of the Museum of Neuchâtel by Jeannet (1929). The illustration of the cast provided by Cottreau (1914) was utilized in the discussion (see Figs. 103-104).

OTHER EXAMINED MATERIAL. A specimen (MNHN-F A22706-L18.471), collection Lambert; Figs. 25, 26, Figs. 100-102, 105 from the late Aquitanian-early Burdigalian of Carry-Le-Rouet (Bouches-du-Rhône).

DESCRIPTION. Middle-sized species (mean TL=84.5 mm), with subcircular to elliptical outline (mean TW=104% TL). Only shallow sinuosities, no deep notches, are present on the margin in correspondence of the anterior ambulacra. Test high in the holotype (TH=17% TL). Margin rather thick. Internal structure: in the radiograph (Fig. 76) the shape of the central cavity is not clearly visible, however it seems roundish. The internal support system is well developed and highly complicated especially in the anterior half of the test, it is denser around the lunules and towards the posterior test margin. The spaces between the pillars are wide, as in *A. montezemoloi* (Fig. 85). Apical system slightly eccentric anteriorly (mean L4=56% TL), large (about 9% TL). Petalodium size variable (PL=52-60% TL). Frontal petal long (L5=25-30% TL), L6=12.5-16% TL. Width of poriferous and interporiferous areas=3.5-4.8% TL and 5.1-6.4% TL, respectively. Anterior paired petals are 24% TL long and 13% TL wide. In the posterior petals, L9=22-27 %TL and L10=13-16% TL. Lunules almost subcircular (mean SI=1.3) and rather small (mean WI=82.2). The distance between the tip of the pos-

terior petals and the corresponding lunules is rather short (range $L3=4-7\%$ TL); the space is occupied by only one or two plates per column. In the oral interambulacrum 5, column “a” includes two post-basiconal plates, three plates are present in column “b”, and there are on the whole 15–16 plates, as well as in the ambulacra I and IV. The length of the oral interambulacral plate 5.b.2 is about 8% TL. Peristome large ($\varnothing ps=4,8\%$ TL), roundish and slightly anterior to the centre ($L12=47-54\%$ TL). Basicoronal circlet rather large ($L13=13-14\%$ TL). Periproct small ($\varnothing pc=2,2\%$ TL), not far from the posterior test margin ($L11=7\%$ TL) and positioned halfway along the suture between plates 5.a.2–5.b.3. Food grooves not well visible, however it seems that they develop by a simple scheme and do not reach the margin; a short branch of the posterior grooves surrounds the lunules.

REMARKS. *Amphiope elliptica* differs from *A. bioculata* by the lack of notches along the margin in correspondence of the anterior paired ambulacra, by the higher number of plates in the posterior ambulacra and a different internal structure, with its roundish central cavity instead of sub-pentagonal. It differs from *A. lorioli* by the internal structure, which is more reduced almost in the whole test (compare Fig. 76 with Fig. 86), by its smaller lunules, which are roundish instead of ovaloid, its higher test and above all a much larger petalodium ($PL=52-60\%$ TL, against 44–46% TL). *A. ovalifora* has ovaloid lunules, as well as *A. lovisatoi*, which has also a higher value of $L4$ (mean $L4=62\%$ TL, against 57% TL). *A. elliptica* differs from *A. ludovici* in having a larger and higher test, higher whole number of plates in the ambulacrum I (15–16 against 11–12). *A. montezemoloi* has much larger lunules (WI ranges from 200 to 400, mean 330, instead of 82). *Amphiope romani* n. sp. lacks the sinuosities on the margin in correspondence of the anterior paired and odd ambulacra, has less numerous plates in the interambulacrum 5 and a different internal structure.

Based on its stratigraphical and geographical position, *A. elliptica* likely represents a link between *Amphiope* from the Burdigalian of south-eastern France and the species subsequently diffused in Corsica, Sardinia and Italy mainland, e.g. *A. “bioculata”* of Aleria (Corse), described in Cottreau (1914) and that we’ll redescribe in a subsequent work.

DISTRIBUTION. Carry-le-Rouet (Bouche du Rhône), late Aquitanian-early Burdigalian. After Lambert, 1907) also S. Restitute, Drôme (Langhian-Serravallian), but this citation needs verification.

Amphiope ovalifora Des Moulins in Fallot, 1903 (Figs. 29, 30; Figs. 106–111; Fig. 72; Fig. 78

1837. *Scutella bioculata* Var. B. foraminibus transversé ovatis Nob. Des Moulins: p. 226

1847. *Lobophora bioculata* Var. B - Agassiz in Agassiz & Desor: p. 78

1874. *Amphiope ovalifora* Desmoulins - Benoist: p. 449

1903. *Amphiope ovalifora* des M. - Fallot: p. 82 (pars)

1907. *Amphiope ovalifora* Desmoulins (in Fallot, 1903) – Lambert: pp. 55–56

1927. *Amphiope ovalifora* Desmoulins (in Benoist) - Lambert: pp. 39–44.

TYPE LOCALITY. Gornac, near Bordeaux (Gironde-France). Aquitanian (Burdigalian?).

TYPE MATERIAL. One syntype (as indicated in the original label), consisting of a complete specimen (MNHBx 2014.6.180.1) from Gornac.

OTHER EXAMINED MATERIAL. Thirteen whole specimens (MNHN-F A 22710-L 18.447a-o) and a large fragment from the type-locality. A fragment (labeled as syntype MNHBx 2014.6.180.2) from Cazeneuve (Bazadais) but doubtfully assigned to this species. Four specimens and a large fragment (APBA 20151015_204113) from the Aquitanian of St. Avit (Carrière de Préhac, Landes).

DESCRIPTION. Middle-sized species (mean TL=75 mm, range 58–90 mm), with subcircular outline (mean TW=106% TL) and the anterior part restricted starting from the anterior paired ambulacra. Test of medium height (mean TH=14% TL) (Figs. 110, 111). Margin rather thick, with only shallow sinuosities, no deep notches, in correspondence of the anterior ambulacra. On the oral face, middle ambulacral areas with a shallow and broad depression. Internal structure: central hollow sub-pentagonal (Fig. 78; Fig. 109), its length, from the rear wall to the front, equals about 40% TL. Four main cavities branch from the central hollow: the first, containing the *caecum*, runs along the interambulacrum 2, other two correspond to the interambulacra

3 and 4, the last one being shorter than the other. The fourth cavity extends along the interambulacrum 5, leading to the periproct. The internal ballast system is dense and becomes almost massive, and crossed only by micro-canals, towards the margin. The geometric shapes within the central cavity (Fig. 78) are due to calcite re-crystallization occurred during fossilization and have no diagnostic value. Apical system slightly anteriorly eccentric (mean $L4=54\%$ TL), large (9% TL). The petalodium (mean $PL=50\%$ TL) increases in size during growth: $PL=45$ and 54% TL in specimens with $TL=58$ and 79 mm, respectively (Fig. 64). Paired anterior petals shorter than the frontal one ($L7=84\%$ $L5$); mean $L5=24.5\%$ TL against $L9=21\%$ TL. On the average, in the frontal petal $L6=60\%$ $L5$, in the posteriors $L10=70\%$ $L9$. Interporiferous zones slightly wider than a poriferous one. In the frontal petal $L6=60\%$ $L5$, in the posteriors $L10=70\%$ $L9$. Interporiferous zones slightly wider than a poriferous one. In the oral interambulacrum 5, column "a" is made of two plates (sometime also a part of the third plate), column "b" is made of three plates, with 2b very elongate (up to 15% TL). On the whole, there are 14–16 plates in each column of the interambulacrum 5. Basicoronal circlet well developed ($L13$ up to 20% TL); the size of the basicoronal interambulacral plates is up to 27% TL. The length of the plate 1 in the interambulacrum 5 is up to 14% TL. Lunules transverse elliptical to sub-circular (mean $SI=1.6$, range $SI=1.3–1.9$), they are never narrow and their edges are never parallel. Lunules may be irregularly shaped and different even in the same specimen. Size much variable (range $WI=93–205$, mean $WI=125$). The space between the tip of posterior petals and the corresponding lunules ($L3=6.5\%$ TL) is occupied by only one or two plates per column. Peristome middle-sized ($\varnothing ps=3–5.2\%$ TL, mean 4) and subcentral ($L12=51\%$ TL). Periproct small (mean $\varnothing pc=$ about 2% TL), opening rather close to the posterior margin (mean $L11=9.6\%$ TL, range $7–11.5\%$ TL) near the conjunction of plates 5.a.2-5.b.2-5.a.3. Aristotle's lantern: the radius of a single wing (Mooi, 1989) is about 12% TL. Food grooves well marked and developed by a simple scheme (Fig. 72), though sometimes they are well branched distally. Secondary ramification almost missing along the middle part of the main grooves. A short branch of the posterior grooves surrounds the lunules and extends towards the rear of the periproct.

DISTRIBUTION. This species seems common in south-western France, above all in the Departments of Gironde and Landes. Faluns de Gonin near Gornac (Lambert, 1926). It was reported also from Cabaron, near Castelvieuil, but this citation needs verification. Cited by Fallot (1903) at Sainte Croix-du-Mont, Château du Cros near Louplac, Garries near Cabanac and Cazeneuve, in the Bazadais. Also S.t Avit (Carrière de Préhac), Landes (personal communication, F. Meunier, APBA Bordeaux). All these records were dated to the Aquitanian.

REMARKS. *Amphiope ovalifora* differs from *A. bioculata* by its lower test (Fig. 54), and from *A. lovisatoi* and *A. bioculata* by higher values of $L13$, lower values of SI and WI , and a denser internal ballast system, above all towards the margin (Figs. 77, 78 and Fig. 109). Differs from *A. lovisatoi* also by a higher value of $L11$, a lower mean value of $L4$ (Fig. 88) and a denser internal structure. *Amphiope elliptica* has roundish lunules and the internal structure is much more reduced and more complicated, also towards the periphery (Figs. 76, 78). Also *A. lorioli* has a much lighter internal structure (Figs. 109, 113). *Amphiope ludovici* has less numerous plates in the interambulacrum 5 and in the ambulacra I and V. *Amphiope montezemoloi* has much larger lunules with roundish shape (WI ranges from 200 to 400, mean 330) (Fig. 53). *Amphiope romani* n. sp. lacks notches in correspondence of the paired anterior ambulacra and the value of $L11$ is very low (Fig. 52). The specimen attributed to *A. ovalifora* figured by Fallot (1903) from Cazeneuve (Bazadais) (not Des Moulins collection), some of those from Prehac and those with narrow lunules from Le Thil examined in Lambert collection (MNHN-F), likely do not belong to this species. Also the specimens from the Aquitanian of Castelvieuil (Cabaron) differ from the type series of *A. ovalifora* from Gornac, by the lower height (mean $H=8\%$ TL, against 14% TL).

Amphiope lorioli Lambert, 1907 - Figs. 31, 32; Figs. 112–115

1902. *Amphiope perspicillata* (non Agassiz) - de Loriol: p. 23; pl. 3, figs. 2, 3

1907. *Amphiope lorioli* Lambert: p. 56.

TYPE LOCALITY. St-Félix-de-Lodez (Lodéne or Lodève *auctorum*) (Hérault - France). Tortonian (Roman, 1974).

TYPE MATERIAL. A complete specimen (MNHN-F A22707-L 18472Aa) from the type locality.

OTHER EXAMINED MATERIAL. A specimen (MNHN-F.A22707-L 18472Ab) from the type locality).

DESCRIPTION. Middle-sized species (mean TL=84 mm), with elliptical to sub-circular outline (TW=111% TL). Test rather low (mean TH=11.7% TL). Margin with slightly deep notches in correspondence of the anterior ambulacra. Internal structure: the central hollow is vaguely pentagonal-starry shaped, with the rear wall lying between the two posterior paired ambulacra and located at the right side of the interambulacrum 5. From the rear wall to the front, the length of the size of the hollow equals 44–48% TL. Five long and wide extensions, starting from the central hollow, extend along the interambulacra. The internal buttress system is reduced, it becomes denser only towards the test margin. Apical system medium-sized (7.5–8.5% TL) and eccentric anteriorly (mean L4=55% TL). Petalodium small to medium-sized (mean PL=45% TL, range 44–46% TL). Frontal petal longer (L5=24.5% TL; L9=18.5 %TL) and proportionally narrower than the posteriors (L6=12.5% TL, L10=12% TL). The mean width of poriferous and interporiferous areas is 4% TL and 5% TL, respectively. Lunules mid-sized (mean WI=130% TL), slightly transversely ovoidal (mean SI=1.4). The space between the tip of posterior petals and the corresponding lunules (L3=7% TL) is occupied by two or three plates per column. In the oral interambulacrum 5, column “a” is made of two (sometimes also a part of the third) plates, column “b” of three. The basicoronal interambulacral plate 1 seems short (about 6% TL). On the whole there are 15–16 plates in each column of the ambulacra I and V and in the interambulacrum 5. Peristome small (mean ϕ ps=3.6–4% TL) and centrally located (mean L12=52%TL). Periproct small (ϕ pc=2%TL), far from the posterior margin (mean L11=12.5%TL, range 10–15% TL), opened between plates 5.b.2–5.a.2 in the studied sample. Food grooves not well visible, however they develop by a simple scheme and do not reach the margin; a short branch of the posterior grooves surrounds the lunules and extends towards the periproct.

DISTRIBUTION. Serravallian-Tortonian of S. Felix de Lodez (Hérault, France). Probably also the

Langhian-Serravallian of La Crucca and Porto Torres (Sardinia, Italy) (see Stara et al., 2012).

REMARKS. *Amphiope lorioli* differs from *A. bioculata* by a different internal structure, with smaller central hollow, and internal ballast system that becomes denser towards the test margin. It differs from *A. ovalifera* by smaller petalodium (PL=45% TL, against 50% TL), less elliptical lunules and different internal structure. It differs from *Amphiope lovisatoi* by the lower value of L4 (mean L4=55% TL, against 62) and PL (45% TL, against 53%TL). *Amphiope ludovici* has smaller and lower test, lower number of plates in each column of the interambulacrum 5 (11–12 against 15–16). *Amphiope lorioli* is distinguished from *A. montezemoloi* by smaller test and lunules (WI=130 against 330) and denser internal structure. *Amphiope romani* n. sp. lacks notches along the margin in correspondence of the anterior ambulacra, has a different internal structure and lower distance of the periproct from the margin, greater petalodium (PL=53%TL, against 45% TL) and a lower number of plates in the oral interambulacrum 5.

Amphiope ludovici Lambert, 1915 - Figs. 33, 34; Figs. 116–120

1912a. *Amphiope elliptica* Desor - Lambert: p. 77; pl. 6, figs. 1–3

1915a. *Amphiope ludovici* Lambert: p. 220; pl. 6, fig. 1a; pl. 16, figs. 14, 15.

TYPE LOCALITY. Blanqui, near Cucuron (Vaucluse - France). Tortonian (Roman, 1974).

TYPE MATERIAL. Two syntypes (MNHN-F J00999 L18473, L18474), from the type locality.

DESCRIPTION. Very small-sized species (mean TL=29 mm). The specimens under study are adults since they have four open gonopores (Fig. 119). Test sub-circular, wider than long (mean TW=108% TL) (Figs. 116, 117). The test is domed and low (mean TH=9% TL) with the highest point anterior to the apical disc (Fig. 118). Margin sharp, with only shallow sinuities, no deep notches, in correspondence of the anterior paired ambulacra. Oral face flat. Internal structure: central hollow sub-pentagonal (Fig. 120). From the rear wall to the front, the length of the hollow is about 49% TL. Three short macrocanals extend from the central

hollow, along the interambulacra 2, 3 and 4; another long channel runs along the interambulacrum 5 and leads to the periproct. Internal ballast system well developed in the whole test. Apical system eccentric anteriorly ($L4=59\%$ TL) and broad (10% TL). Petalodium medium-sized ($PL=50\%$ TL). Anterior petal longer than the posteriors ($L5=25\%$ TL against $L9=22.4$; $L7=24\%$ TL); the width of the anterior and the posterior petals is comparable ($L6=14\%$ TL, $L10=13.5\%$ TL; $L8=14\%$ TL). Width of interporiferous and poriferous zones= 6.4% and 4.2% TL, respectively. In the oral interambulacrum 5, the length of the basicoronal plate 1 is 9% TL, that of plate 5.b.2 is 16% TL. There are two post basicoronal plates in column "a" and two, seemingly three, in column "b". In the interambulacrum 5 there are, on the whole, only 10–11 plates per column. In the ambulacrum I there are 11–12 plates per column (Figs. 33, 34). Lunules rounded (mean $SI=1.1$) and small-middle-sized (mean $WI=100$). The space between the tip of posterior petals and the corresponding lunules ($L3=7\%$ TL) is occupied by only 1–2 plates per column. Peristome eccentric anteriorly ($L4=59\%$ TL); $\varnothing ps=4.5\%$ TL. Periproct small ($\varnothing pc=1.6\%$ TL), very close to the posterior margin ($L11=4.6\%$ TL) and positioned between plates 5.a.2–5.b.3. Food grooves partially visible, likely developing by a simple scheme and not reaching the margin.

DISTRIBUTION. Blanqui near Cucuron (Vaucluse - France), Tortonian.

REMARKS. *Amphiope ludovici* differs from *A. bioculata*, *A. elliptica* and *A. lorioli* by the lower number of plates in each column of the posterior ambulacra and in the interambulacrum 5. *Amphiope ovalifora* has a different internal structure, more transversely elongate and larger lunules and much higher test. *Amphiope ludovici* differs: from *A. lovisatoi* by its rounded lunules and a lower value of $L4$ (mean $L4=55\%$ TL against 62), from *A. montezemoloi* by much smaller lunules ($WI=88$ against 330), from *A. romani* n. sp. by the presence of notches on the test margin, the different internal structure and the lower number of plates in the interambulacrum 5 and the posterior ambulacra. It is probable that the specimens from Cucuron (les Castellas) assigned to *A. baquiei*, examined at the MRA, belong to *A. ludovici*. Unluckily it was not possible to detect the plating structure.

Amphiope romani n. sp. - Figs. 121–124; Figs. 161, 163

1915. *Amphiope bioculata* (var. *turonensis*) Desmoulins - Lambert: p. 77

2014. *Amphiope* sp. 3 Stara & Sanci: pp. 318, 320; pl. 1, figs. 1–7; pl. 2, figs. 1–6.

TYPE LOCALITY. Faluns of Channay-sur-Lathan, Touraine (France), late Serravallian-early Tortonian (after André et al., 2003).

TYPE MATERIAL. Holotype (MNHN-F.A57780.PL1669), represented by a complete specimen, and a paratype (MNHN-F.A57781.PL1821).

OTHER EXAMINED MATERIAL. 16 specimens, 12 of them (MNHN-F.A57781.PL1821–6; MNHN-F.A57781a–f; MACPL 1668) from the late Serravallian-early Tortonian of Channay-sur-Lathan (Touraine - France) and four (MNHN-F.A.22713–L18482a, b; MNHN-F.R7277a, b, f) from Oisly (Loir-et-Cher - France). The outcrops of Oisly were attributed to the Langhian-Serravallian by André et al. (2003).

DIAGNOSIS. A species of *Amphiope* characterized by a middle-sized test with sub-circular outline, margin thin and almost deprived of notches, lunules rounded, separated by only one or two (rarely three) plates from the tips of the posterior petals and rather close to the rear margin; internal ballast system very light and becoming very dense near the margin.

DESCRIPTION OF HOLOTYPE. Test middle sized ($TL=68$ mm), with subcircular outline ($TW=108\%$ TL). Margin rather thin, lacking notches in correspondence of the anterior ambulacra. Test of middle height ($TH=13.2\%$ TL) (Fig. 122). Oral face with interambulacral areas slightly inflated and ambulacral areas with a shallow and broad depression deepening towards the peristome. Internal structure: central hollow sub-pentagonal to star-shaped (Fig. 121). From the rear wall to the front, the length of the hollow is about 49% TL. A long and wide macrocanal extends from the central cavity along the interambulacrum 2; another long and narrow channel runs along the interambulacrum 5 and leads to the periproct. The internal buttress system is very reduced, becoming densely packed near the test margin. Apical system an-

teriorly eccentric ($L4=59\%$ TL), large (about 10% TL). Petalodium large ($PL=53\%$). Paired posterior petals shorter than the frontal one ($L5=26,5\%$ TL, $L9=24,3\%$ TL). The width of the petals is comparable ($L6$ and $L10=15\%$ TL). Width of an interporiferous zones 1.2–1.5 times than that of a poriferous one. In the oral interambulacrum 5, column “a” is made of two plates, column “b” is made of two plates. The length of plate 5.b.2 is $16,4\%$ TL. On the whole, there are 14 plates per column in the interambulacrum 5 and 13–14 in the ambulacra I and V. Basicoronal circlet large ($L13=14\%$ TL); the length of interambulacral plate 1 is about 9.5% TL. Lunules mid-sized ($WI=121,5$), transverse elliptical ($SI=1.5$). The distance between the tip of posterior petals and the corresponding lunules is long ($L3=7\%$ TL) and is occupied by two plates per column. Peristome middle-sized ($\varnothing ps=4\%$ TL) and opening centrally ($L12=50\%$ TL). Periproct small ($\varnothing pc=1.7\%$ TL), very close to the posterior margin ($L11=6,7\%$ TL) and located between plates 5.a.2–5.b.3. Food grooves well marked, developing by a simple scheme but well branched distally; they almost reach the margin; a short branch of the posterior grooves surrounds the lunules and proceeds towards the periproct, not reaching it.

VARIABILITY. Middle sized species: the test length ranges from 46.5 to 73 mm, the test height ranges from 13 to 16.5% TL (mean $TH=14.5\%$ TL) in the studied sample. Margin lacking notches or with only shallow sinuosities in correspondence of the anterior ambulacra. The internal buttress system is always very reduced, becoming densely packed near the test margin. Apical system anteriorly eccentric (mean $L4=58\%$ TL). Petalodium size variable, ranging from 51 to 62% TL (mean 54% TL). Also petals variable in size; paired posterior shorter than the frontal one (mean $L5=26\%$ TL, $L9=24\%$ TL). The width of the petals is almost comparable (mean $L6$ and $L10=15\%$ TL). In the oral interambulacrum 5, column “a” is always made of two plates, column “b” is made of two plates, sometimes with also a part of the third plate. The whole number of plates is constant: 13–14 plates per column in the interambulacrum 5 and 12–13 in the ambulacra I and V. Basicoronal circlet large (mean $L13=12.5\%$ TL). Lunules small to mid-sized (mean $WI=123$, range 90–157), commonly rounded to transverse elliptical (mean

$SI=1.2$, range 1–1.6). The distance between the tip of posterior petals and the corresponding lunules is occupied by one or two (rarely three) plates per column. Periproct close to the posterior margin (mean $L11=5.2\%$ TL, range 3– 10% TL) and located between plates 5.a.2–5.b.3 or at the conjunction of plates 5.a.2–5.b.2–5.b.3.

DERIVATIO NOMINIS. This new species is dedicated to the French geologist, paleontologist and echinologist Jean Roman.

DISTRIBUTION. The species is common in the late Serravallian-early Tortonian Faluns of Channay-sur-Lathan (Touraine, France); a variety is present in the Langhian-Serravallian of Oisly (Loir-et-Cher, France).

REMARKS. The specimens from Oisly differ from those from Channay-sur-Lathan by higher test, larger lunules, smaller petalodium and by the presence of shallow notches along the test margin in correspondence of the paired anterior ambulacra. Given the small sample available for the study and the different stratigraphic position of the *Amphiope*-bearing sediments at Channay and Oisly, the subspecies *A. romani turonensis* (Lambert, 1915), established for the specimens from Oisly, is maintained. *Amphiope romani* n. sp. is distinguished mainly by the periproct very close to the posterior test margin (with the exception of *A. elliptica* and *A. ludovici*) and by the lack of notches on the margin in correspondence of the anterior ambulacra. *A. elliptica* has a greater whole number of plates in the interambulacrum 5 (15–16) than *A. romani* n. sp. (12–14) and *A. ludovici* (only 10–12). *Amphiope romani* n. sp. has a different internal structure when compared with *A. elliptica* and *A. ludovici*. *Amphiope montezemoloi* has much larger test and lunules.

The *Amphiope nuragica* group (sensu Stara et al., 2015)

This informal group includes the species with transversely elongate lunules and values of $SI > 1.6$: *A. hollandei*, *A. depressa*, *A. palpebrata*, *A. sarasini*, *A. transversifora*, *A. deydieri*, *A. pallavicinoi*, *A. tipasensis*, *A. dessii* and *A. nuragica*. Remarks about *A. depressa* and *A. palpebrata* are reported in

the discussion chapter, for *A. nuragica* see Stara & Borghi (2014).

Amphiope hollandei Cotteau 1877 - Figs. 37, 38; Figs. 125–128

1877. *Amphiope hollandei* Cotteau: p. 241; pl. 9, figs. 6, 7; pl. 20, fig. 1

1907. *Amphiope hollandei* Cotteau - Lambert: p. 57

1925. *Amphiope hollandei* Cotteau - Lambert & Thiéry: p. 122

1998a. *A. bioculata* (Desmoulins) - Philippe: p. 152.

TYPE LOCALITY. Bonifacio (Corse - France), Burdigalian.

TYPE MATERIAL. Holotype (MNHN-F.A22378. L18472).

DESCRIPTION. Large-sized species (TL=106 mm). Test ovoid, much wider than long (TW=120% TL). The test is domed and very low (TH=6.5% TL). Margin thick, with rather deep notches in correspondence of the anterior paired ambulacra. Oral face flat. Apical system sub-central (L4 about 57% TL). Petalodium rather wide (PL=54% TL). Frontal petal longer (L5=30% TL, L9=24% TL) and wider than the posteriors (L6=17.5% TL, L10=16 %TL). The width of the interporiferous and poriferous zones is 8.3% TL and 4.7% TL, respectively. The plating pattern in the interambulacrum 5 is partially visible. There are three plates in column “a” and, at least, three in column “b”. In the aboral ambulacrum V there are eight or nine plates per column around the lunules, pointing to the presence as a whole of 14–15 plates per column. Lunules transverse, long and narrow L1=3.2% TL (SI=7.3, WI=72). Peristome slightly anterior to the centre (L12 about 60% TL). Periproct rather far from the posterior margin (L11 about 12% TL), opening at the conjunction of plates 5.a.2-5.b.2-5.b.3.

DISTRIBUTION. Bonifacio (Corse - France), Burdigalian.

REMARKS. *Amphiope hollandei* is distinguished mainly by its very low test and the particular shape of the lunules, which are very long and narrow (SI up to 7, against a maximum SI= 3 in *A. nuragica*).

Amphiope sarasini Lambert, 1907 - Figs. 43, 44; Figs. 129–133

1902. *Amphiope perspicillata* (non Agassiz) - de Loriol: p. 23; pl. 3, figs. 2, 3

1915. *Amphiope sarasini* Lambert: p. 222; pl. 4, figs. 8, 10; pl. 3, fig. 24.

TYPE LOCALITY. Cruzy (Hérault - France). Seravallian-Tortonian, after Roman (1974).

TYPE MATERIAL. A syntype (MNHN-F J00985.L18480), consisting of a complete specimen from the type locality.

OTHER EXAMINED MATERIAL. Three specimens (MNHN-F.A22379 L18.469; MNHN-F.A.57788-89) from the type locality.

DESCRIPTION. Middle-sized species (TL=60-93 mm in the studied sample), with elliptical outline (TW=108.5% TL). Test with middle-height (mean TH=14% TL). Margin with shallow notches in correspondence of the anterior paired ambulacra. In the oral face the anterior ambulacral areas and the first half of the interambulacra are distinctly sunken (Fig. 133). Internal structure: the central hollow is sub-pentagonal to starry shaped. From the rear wall to the front, the length of the hollow ranges from 44 to 48% TL. Ten long and wide extensions, starting from the central hollow, extend in correspondence of the interambulacra. The peripheral ballast system is of middle density and becomes stronger only towards the test margin. Apical system medium-sized (8-10% TL) and eccentric anteriorly (mean L4=57% TL). Petalodium increases in size with growth (range PL=46-52% TL; mean PL=49% TL). The mean width of the frontal and the posterior petals is comparable: L6=13% TL, L10=14% TL. The mean width of poriferous and interporiferous areas is 4% TL and 5.2% TL, respectively. Lunules small (mean WI= 97% TL), transversely ovoidal (mean SI=1.8). The distance between the tip of posterior petals and the corresponding lunules (L3= 7% TL) is occupied by two plates per column. In the oral interambulacrum 5, column “a” is made of two or three post-basicoronal plates, column “b” of three. The basicoronal interambulacral plate 1 is short (about 6% TL). On the whole, there are 13–15 plates per column in the interambulacrum 5 and 16–17 in the ambulacra I and V. Peristome small (mean σ ps=3.4–4% TL) and centrally located (mean

L12=51% TL). Periproct small (\varnothing pc=1.8% TL), far from the posterior margin (mean L11= 12, range 10–13% TL) and opened between plates 5.a.2–5.b.3 or 5.a.2–5.b.2–5.b.3. Food grooves well marked and strongly branched distally; numerous short secondary ramifications branch off also long the main grooves.

DISTRIBUTION. Serravallian-Tortonian of Cruzy.

REMARKS. *Amphiope sarasini* is distinguished from the other species belonging to the *A. nuragica* group by its very small lunules, the smallest so far observed in *Amphiope*, the distinctly sunken oral anterior ambulacral areas and the food grooves, which are the most strongly branched in the genus *Amphiope*. This species is apparently close to *A. depressa*, however the plating patterns and the internal structure of the last species are unknown, thus preventing a reliable comparison.

***Amphiope transversifora* Lambert, 1912**

Figs. 39, 40; Figs. 134–137

1912a. *Amphiope transversifora* Lambert: pp. 84–85; pl. 7, figs. 3–5

1925. *Amphiope transversifora* Lambert - Lambert & Thiéry: p. 122.

TYPE LOCALITY. Saint-Paul-Trois-Châteaux (Drôme - France), Langhian.

TYPE MATERIAL. A syntype (MNHN-F.J01682), represented by a slightly damaged specimen, from the type locality. The other syntypes cited by Lambert (1912a) are wanting.

DESCRIPTION. Small-sized species (TL=46 mm). Test sub-circular, wider than long (TW=108% TL). The test is domed and rather low (TH=13% TL). Margin thick, with rather deep notches in correspondence of the anterior paired ambulacra. Oral face flat. Internal structure: central hollow roundish, its size is not measurable because the perioral area is damaged. The internal buttress system is reduced, with sparse supports and large spaces. Also the peripheral ballast system is reduced and becomes densely packed only close to the test margin. Apical system slightly eccentric anteriorly (L4=58% TL), broad (9% TL). The only specimen available to study has three small gonopores. Petalodium large (PL=55% TL).

The frontal petal is longer than the posteriors (L5=27% TL, L9=24% TL); the width of the petals is almost proportionally comparable (L6 and L10= 15% TL). Width of the interporiferous and poriferous zones 5.8–7.2% TL and 4.1–4.8% TL, respectively. In the oral interambulacrum 5 there are on the whole about 12–13 plates per column; adorally there are only two post-basical plates in column “a” and two, plus a small part of the third, in column “b”. The length of plate 5.b.2 is 18% TL. Lunules transversely elongate, rather narrow (mean SI=2) and medium sized (WI=149). The distance between the tip of posterior petals and the corresponding lunules (L3= 6.5% TL) is occupied by two plates per column. Peristome slightly anterior to the centre. Periproct small (\varnothing pc=2.4% TL), rather close to the posterior margin (L11=7.4% TL) and positioned near the conjunction of plates 5.a.2–5.b.2–5.b.3. Posterior food grooves partially visible, it seems that they develop by a simple scheme.

DISTRIBUTION. Saint-Paul-Trois-Châteaux (Drôme - France), Langhian.

REMARKS. *Amphiope transversifora* is distinguished mainly by the presence of only two post-basical plates in each column of the oral interambulacrum 5, the lowest in the *A. nuragica* group. It differs from the apparently similar *A. nuragica* by its delicate internal structure, from *A. sarasini* by its lunules, which are larger and much closer to the posterior test margin.

***Amphiope deyrieri* Lambert, 1912**

Figs. 41, 42; Figs. 138–142

1912a. *Amphiope deyrieri* Lambert: pp. 85–86; pl. 6, figs. 5, 7.

TYPE LOCALITY. Cadenet, near Vaugines (Vaucluse - France). Serravallian.

TYPE MATERIAL. Holotype (MRA 3.000.157). The type series studied by Lambert (1912) consists also of 10 whole specimens (MRA3000.159–162, MRA 3000-164; MNHN-F A22705–L18470a-d), from the type locality.

OTHER EXAMINED MATERIAL. Six additional specimens (MRA) from the type locality, not belonging to the series studied by Lambert.

DESCRIPTION. Small-sized species (TL=49-53 mm). Test wider than long (mean TW=122% TL), with the anterior part restricted. Test domed (mean TH=12%; range 10-16% TL). Margin sharp, with shallow notches in correspondence of the anterior ambulacra. Oral face slightly concave, with interambulacral areas slightly inflated and the median ambulacral areas with a shallow and broad depression. Internal structure: though the X-ray photograph is not very clear (Fig. 140), due to the high density of the material, the central hollow is broad and vaguely roundish in shape. A large cavity extends from the central hollow through the interambulacrum 2, another one leads to the periproct. Internal buttress system reduced, with large spaces towards the test margin. Apical system medium sized (8.3 % TL), slightly eccentric anteriorly (L4=56% TL), with small gonopores. Petalodium large (mean PL= 53% TL). Posterior petals shorter than the others (mean L5=27% TL against L9=23 %TL. The petals width is similar: L6=15% TL, L10= 15.5% TL. Interporiferous zones slightly wider than a poriferous one. Basicoronal circlet large. In each column of the interambulacrum 5 there are on the whole 10-11 plates, only two post-basicoronal plates are present adorally in each column. Lunules narrow transverse, ovate or slit-like (mean SI=2.3), always mid-sized (mean WI=113.5). They are separated from the posterior petal tips by two plates per column (L3= 5.3% TL). Peristome sub-central (mean L12=56%TL) and mid-sized (\emptyset ps= about 4.4% TL). Periproct small (\emptyset pc=2.8% TL), located at the conjunction of plates 5.a.2-5.b.2-5.b.3. Distance from the posterior margin much variable (range L11=6-13% TL). Food grooves well marked, developing by a simple scheme, not reaching the margin.

DISTRIBUTION. Cited only from the Langhian-Serravallian of Cadenet, near Vaucluse - France).

REMARKS. *Amphiope deyrieri* is distinguished mainly by its transversely elongate test: it has the highest value of TW in this group (TW=122% TL). Only *Amphiope hollandei* has a similar test shape (TW=120% TL), however the shape of the lunules in this species is much different (SI=2.3 against 7). *Amphiope deyrieri* differs from *A. sarasini*, *A. transversifora* and *A. nuragica* by the lower whole number of plates in the interambulacrum 5 and am-

bulacra I e V. *A. sarasini* is distinguished from *A. deyrieri* also by its much smaller lunules, *A. transversifora* by its much more reduced internal structure.

***Amphiope pallavicinoi* Lovisato, 1914**

Figs. 143-147; Figs. 151, 152

1914. *Amphiope pallavicinoi* Lovisato: p. 115; pl. 2, fig. 5a-b.

TYPE LOCALITY. Lovisato (1914) indicated Torralba (Sassari Province, Sardinia) as type-locality. The type-stratum crops out also at Bessude, near Torralba, 40°33'18.94"N , 8°43'11.33"E. late Burdigalian.

TYPE MATERIAL. The type material of *A. pallavicinoi* is wanting and no other specimens from Torralba, the type locality indicated by Lovisato (1914), are known. We were able to collect new specimens from the type stratum which crops out also at Bessude, some 6 km far from Torralba. A neotype is here proposed (MDLCA 23583); it is represented by an almost complete specimen (TL=100 mm), from Bessude.

OTHER EXAMINED MATERIAL. A fragment from Bessude (MAC.PL1474), a specimen photographed in situ and a figure published by Lovisato (1914: pl. 2, fig. 5a).

DESCRIPTION. Large-sized species (TL=100-105 mm in the studied sample). Test rounded, as long as wide (TW=111 % TL). Test low (range TH=9-13% TL). Margin relatively thick, with notches in correspondence of the anterior ambulacra. Internal structure: the visceral hollow is sub-pentagonal to starry shaped. From the rear wall to the front, the length of the hollow measures 46% TL. Two long extensions, starting from the central hollow, extend into the interambulacra 1 and 5. The peripheral buttress system is reduced and becomes denser only rear to the lunules, towards the test margin. Apical system large (range 10-11.5% TL), located far from the posterior margin, range L4= 58-66% TL). Petalodium large (PL=56-62% TL). Petals almost equal in size: L5=28% TL, L7=27-28% TL, L9=26-30 %TL. Petal width variable: L6, L8 and L10 range from 16 to 18% TL. Interporiferous zones similar or slightly wider than a poriferous one, not rising over the test surface. Ba-

sicoronal circlet not detectable. In the oral interambulacrum 5 there are likely two plates in column a and three in column b. In the ambulacra I and V there are on the whole 15–16 plates. Lunules narrow (mean SI= 2.45) and middle sized (mean WI=151). They are separated from the posterior petal tips by one or two plates per column (L3=4% TL) and their distance from the rear margin equals 14% TL. Peristome anterior to centre (L12=65%TL) and large (ϕ ps=5.6% TL). Periproct small (ϕ pc=1.9% TL), located at the conjunction of plates 5.a.2–5.b.3. Its distance from the posterior margin is short (mean L11= 9% TL). Food grooves not well visible; they seem to develop by a simple scheme and do not reach the margin.

DISTRIBUTION. Late Burdigalian of Torralba (Lovichato, 1914) and Bessude, Sassari Province (Sardinia, Italy).

REMARKS. *Amphiope pallavicinoi* is distinguished mainly by its large but low test and large petalodium; *Amphiope tipasensis* has roundish test outline and higher whole number of plates in the ambulacra (17 against 14) and smaller lunules. *Amphiope nuragica* has the highest number of plates in this group. At equal size, *A. sarasini* has smaller lunules, *Amphiope hollandei* has a much wider test (TW=120% TL) and much narrower lunules (WI=7, against 2.4). *Amphiope transversifora* differs by the lower whole number of plates in the interambulacrum 5 and the ambulacra I e V.

Amphiope tipasensis Roman in Aymé et Roman, 1954 - Figs. 148–150; Figs. 153, 154

1954. *Amphiope tipasensis* Roman: p. 168; pl. 1, fig. 1–2.

TYPE LOCALITY. Tipasa Province, Algeria. Pliocene (“Astian” after Aymé & Roman, 1954).

TYPE MATERIAL. Holotype (MNHN-F.R06930).

DESCRIPTION. Medium-sized species (TL=92 mm). Test rounded, as long as wide (TW= 118% TL). Test domed but rather low (TH=13% TL). Margin sharp, with shallow notches in correspondence of the anterior ambulacra. In the oral face, interambulacral areas slightly inflated and the median ambulacral areas with a shallow and broad

depression. Internal structure: not detected. Apical disc not visible. Petalodium small (PL= 46% TL). Petals almost equal in length: L5 \cong 23% TL, L7 \cong 22 % TL, L9 \cong 20 % TL. Also their width is similar: L6, L8, L10= 12% TL. Interporiferous zones similar in size or slightly wider than a poriferous one; they are slightly raised over the test surface. Basicoronal circlet small. In the interambulacrum 5 the length of the basicoronal plate 1 is 6.5% TL. In the oral interambulacrum 5 there are three post-basicoronal plates in column a and four in column b. In the ambulacra I and V there are on the whole 15–17 plates. Lunules narrow transverse, slit-like (mean SI= 2.1) and always small (mean WI=99.3). Foramen with oblique walls. The lunules are separated from the posterior petal tips by only 2 plates per column (L3= 4,8% TL). Peristome central (L12=50%TL) and small. Periproct small (ϕ pc=1.9% TL), located at the conjunction of plates 5.a.2–5.b.2–5.b.3; it is far from the posterior test margin (L11= 12.5% TL). Food grooves well marked, developing by a simple scheme and do not reaching the margin.

DISTRIBUTION. Pliocene of Tipasa Province (Algeria).

REMARKS. *Amphiope tipasensis* is distinguished mainly by its roundish test and the high number of oral interambulacral plates. Only *A. nuragica* has a similar number of plates, but it has larger lunules. At equal size, *A. sarasini* has a larger petaloid and much more complex ramifications in the food grooves. *Amphiope hollandei* has a wider test (TW= 120% TL) and much narrower lunules (WI=7 against 2.1). *Amphiope transversifora* differs by the lower whole number of plates in the interambulacrum 5 and ambulacra I e V. *Amphiope palpebrata* differs in having larger petalodium and lunules and a higher number of plates in the oral ambulacra I and V (compare Figs. 153, 154 and 155, 156).

Genus *Paraamphiope* Stara et Sancier, 2014

TYPE SPECIES. *Paraamphiope raimondii* Stara et Sancier, 2014

TYPE MATERIAL. The holotype (MZE.UNICA-

MAC.IVM206) is represented by a well preserved whole specimen (Recent, Indonesia).

DIAGNOSIS. Emended from Stara & Sanciù (2014):

- Test low with thin margin; highest point corresponding to the centre or slightly anterior to it. Oral side flat to slightly concave.
- Ambulacra a little wider than interambulacra at ambitus. Petals short (about half radial length of test). All five petals similar in length. Petalodium small (42–51% TL) with petals almost closed distally; β angle about 88° .
- Width of the sub-pentagonal visceral central hollow equals almost that of the petalodium. Five cavities branch from the central hollow along the interambulacra; the longest one extends along the interambulacrum 5 and leads to the periproct.
- Apical disc monobasal, subcentral or slightly anterior to centre, with four small gonopores.
- Interambulacra narrower than the ambulacra adorally, even at their widest point. The first two plates in the interambulacrum 5 are slightly staggered with only the plate 5.b.2 in contact with the two first ambulacral post-basicoronal plates.
- Width of the interambulacrum 5 at the margin is almost 36% TL.
- Basicoronal circlet pentastellate with interambulacral plates forming the points.
- Two radially elongate ellipsoidal lunules or narrow slits present in the posterior ambulacra; three or four pairs of plates separate the lunules from the tip of the corresponding petals.
- Peristome small, sub-central or slightly anteriorly located.
- Periproct circular, small, with distance periproct-posterior margin $<13\%$ TL.
- Main food grooves well marked, large and strongly branched distally. They do not reach the margin; the posterior grooves extend towards the periproct. Fine and short secondary grooves branch off also along the middle part of the main grooves. Tube-feet extend also into the interambulacral zones.
- Tuberculation dense, made of very small, perforate and crenulate tubercles which are larger on the oral face.

DISTRIBUTION. France, North Africa and Indonesia, Oligocene to Recent.

REMARKS. *Paraamphiope* differs from *Echinodiscus* in having the first two post-basicoronal plates of the interambulacrum 5 staggered, whereas they are always large and paired in *Echinodiscus*. Additionally, in *Paraamphiope* the contact with the post-basicoronal ambulacral plates in the interambulacrum 5 is the same as that in *Amphiope*, while in *Echinodiscus* and usually in *Sculpsitechinus* both plates 5.a.2 and 5.b.2 are in contact with the adjacent ambulacral postbasicoronals. However, some populations of Recent *Sculpsitechinus* show a certain variability. *Paraamphiope* differs from *Amphiope* by axial lunules, separated by three or four (rarely two) couples of plates from the posterior petals, in the latter they are rounded or transverse and separated from petals tip by only one or two (rarely three) couples of plates. *Paraamphiope* has strongly branched food grooves; that are developed on the entire adoral surface in *Sculpsitechinus*. The petalodium size commonly ranges from 42 to 50% TL in *Paraamphiope*, while it frequently gets up to 60% TL in *Amphiope* and 30–60% TL in *Sculpsitechinus*. *Paraamphiope* differs from *Sculpsitechinus* also by the position of the periproct, which is closer to the rear margin (2.5–13% TL against 11–26% TL).

Species included:

- *P. agassizi* (Des Moulins in Cotteau, 1864), Rupelian, Gironde (France).
- *P. arcuata* (Fuchs, 1882), Miocene of Libya and probably Egypt.
- *P. cherichirensis* (Thomas & Gauthier in Gauthier, 1889), Burdigalian of Tunisia.
- *P. baquiei* (Lambert, 1907), Langhian-Serravallian, south-eastern France.
- *P. raimondii* Stara & Sanciù, 2014, Indonesia (Borneo), Recent.

Species previously assigned to the genus *Amphiope* herein transferred to *Paraamphiope*:

Paraamphiope agassizi (Des Moulins in Cotteau, 1864) - Figs. 157, 158, 162; Figs. 165–170; Fig. 74

1864. *Amphiope agassizi* Desmoulins - Cotteau: p. 103; pl. 14, figs. 3, 5

1870. *Amphiope agassizi* Des Moulins - Tournouer: p. 21
 1903. *Amphiope agassizi* des Moul. - Fallot: p. 82
 1907. *Amphiope agassizi* (Desmoulins in Cotteau) - Lambert: p. 55
 1914. *Amphiope agassizi* Des Moul. - Cottreau: pp. 92, 97
 1926. *Amphiope agassizi* Desmoulins (in Cotteau) - Lambert: p. 36.

TYPE AREA. Includes the localities of S. Albert near La Réole and S. Gemme near Montségur (Gironde, France). “*Couches supérieures du Calcaire ad Astéries*”, Rupelian (Oligocene).

TYPE MATERIAL. Two syntypes, one of them (MHNbX 111. 6-194) from S. Albert, the other (MNHN-F.B23973) from S. Gemme.

OTHER EXAMINED MATERIAL. A topo-typic specimen from S. Gemme (MNHN-F.A22688) and five specimens from Pellegrue (MNHN-F.A22687a-e); Rupelian (Oligocene).

DESCRIPTION. Small sized species (mean TL=46 mm, range TL=37-54.5 mm). Test wider than long (mean TW=110% TL), with the anterior part restricted. Shallow to rather deep notches present on the margin in correspondence of the anterior ambulacra. Test low (mean TH=12.5% TL). Oral face flat, with interambulacral areas slightly inflated and ambulacral areas with a shallow and broad depression deepening towards the peristome. Margin rather thin with rather shallow notches in correspondence of the anterior paired ambulacra. Internal structure: central hollow pentastellate (Fig. 167). From the rear wall to the front, the length of the hollow corresponds to PL. Five cavities branch from the central hollow along the interambulacra; the longest one extends along the interambulacrum 5 and leads to the periproct. The internal buttress system is reduced anteriorly and becomes denser towards the posterior test margin. Apical system slightly anteriorly eccentric (mean L4=55% TL), large (8.6% TL); the anterior left pore is closed in three specimens of five in which it was visible. Petalodium size increasing during growth (mean PL=46% TL, range PL=42-48% TL). Posterior petals shorter than the frontal petal (mean L9=85% L5). Width of frontal petal (L6=50% L5) comparable to that in the posteriors (L10=60% L9). Poriferous zones about

half the width of an interporiferous one. Petals may be open distally. In the oral interambulacrum 5, columns “a” and “b” are made of two or three post-basicoronal plates; plate 5.b.2 has a very broad base and is partially paired to 5.a.2, however it remains in touch with the two post-basicoronal plates of the ambulacra I and V (Fig. 158). Basicoronal circlet large (L13=19% TL, taken from MNHN-F A22688); the length of the interambulacral plate 1 is about 11% TL. Lunules radially elongate, ovoidal (mean SI=1.6), never narrow. The lunules outline may be different even in the same specimen. Size is variable (range WI=32-142, mean WI=106). They are rather far from the tip of the corresponding posterior petal (range L3=7-15% TL), the space is occupied by three to five plates. Peristome middle-sized (ϕ ps=4.5% TL), opening sub-centrally. Periproct middle-sized (ϕ pc=2.3% TL) and close to margin (mean L11=5.6% TL, range L11=2.8-8.2% TL), it opens close to the conjunction of plates 5.a.2-5.a.3-5.b.3. Food grooves: as in the diagnosis of the genus (Fig. 74).

DISTRIBUTION. Rupelian of S. Gemme, Ariège, and Saint Albert (Gironde, France). Cited by Labrie (1904) at Pellegrue and by Tournouer (1870) at Meilhan. Cited by Chauzac & Roman (1994) also in the Chattian of Abesse (southern Aquitaine, France).

REMARKS. *Paraamphiope agassizi* differs from *P. raimondii* by much larger basicoronal circlet, much smaller and less radially elongate lunules. *Paraamphiope agassizi* has tubercles along the perradial sutures as in *P. arcuata*, but differs from it by the higher whole number of plates in the interambulacrum 5 and in ambulacra I and V and the lunules closer to the tips of the posterior petals.

Paraamphiope cherichirensis (Thomas et Gauthier in Gauthier, 1889) - Figs. 175-179; Figs. 89, 90

1889. *Amphiope cherichirensis* Thomas & Gauthier: p. 104; pl. II, figs. 9-11
 1914. *Amphiope cherichirensis* Gauthier - Cottreau: p. 138
 1925. *Amphiope cherichirensis* Gauthier - Lambert & Thiéry: p. 122.

TYPE LOCALITY. Djebel Cherichira, near El Houfia, Kairouan (Tunisia). Burdigalian.

TYPE MATERIAL. Holotype (MNHN-F R62290).

OTHER EXAMINED MATERIAL. A large fragment from the type-locality (MNHN-F-R62288) and an almost complete specimen (MNHN-F R67287) from the Burdigalian of Bou Golrine (Tunisia).

DESCRIPTION. Middle-sized species (max TL=75 mm), wider than long (mean TW=123% TL), with the anterior part restricted. Margin thin, with shallow to rather deep notches in correspondence of the anterior paired ambulacra. Test low (mean TH=10% TL). Internal structure: although the X-ray photograph is not clear, due to the poor preservation of the studied specimen, the central hollow is small, likely equaling the size of the petalodium. Internal ballast system very dense. Apical disc slightly anteriorly eccentric (L4=56% TL) and large (10% TL); structure not visible. Petalodium wide (PL=50% TL). Posterior petals shorter than the frontal petal (mean L9=84% L5). L6=50% L5, L10=46% L9. Poriferous zones about the same width of an interporiferous one. The space between the posterior petal tip and the corresponding lunule (range L3=6-8% TL) is occupied by three or four plates in each column. In the oral interambulacrum 5 (MNHN-F R67287) column "b" is made of three post-basical plates, column "a" at least of two (possibly, also a part of the third). Lunules middle-sized (mean WI=140), radially elongate, ovoidal (mean SI=1.4). The plate arrangement around the lunules is characteristic, with three or four plates in each column separating the tip of each petal from the corresponding lunule (range L3=7.6-9). Peristome small and subcentral (L12≈60% TL). Periproct small, close to the posterior margin, it opens near the conjunction of plates 5.a.2-5.b.2-5.b.3 (taken from MNHN-F R67287). Food grooves well branched and not reaching the margin.

DISTRIBUTION. Burdigalian of Djebel Cherichira valley and Bou Golrine (Tunisia).

REMARKS. *Paraamphiope cherichirensis* differs from *P. agassizi* by its wider test (TW=123% TL against 110) and much wider β angle (mean 100°, against 78°). It is distinguished from *P. arcuata* by

the presence of 5 couples of plates around the lunules on the oral face, instead of 4, and larger petalodium (PL=47-49% TL against 42-47). *Paraamphiope raimondii* has smaller petalodium (PL=42% TL) and more radially elongate lunules. *Paraamphiope baquiei* has much smaller test with different outline, more rounded lunules, larger stoma, lower total number of plates in the posterior ambulacra (13-14 against 14-15) and much smaller petalodium.

***Paraamphiope baquiei* (Lambert, 1907)**

Figs. 159, 160; Figs. 171-174; Figs. 58, 59, 61

1907. *Amphiope Baquiei* Lambert: pp. 56-57; pl. 2

1912. *Amphiope Baquiei* Lambert - Lambert: pp. 83-84; pl. 5, figs. 6, 8; pl. 6, fig. 4.

TYPE LOCALITY. The species was originally described by Lambert (1907) from the "Helvétien" of St-Chrystol, near Nissan (Hérault). The neotype subsequently designated by Lambert (1912) was from Blanqui near Cucuron (Vaucluse - France), Tortonian, after Roman (1974).

TYPE MATERIAL. The "type" described by Lambert (1907) was lost just a short time after the institution of the species. The neotype was available to study at the MRA (3.000.154).

OTHER EXAMINED MATERIAL. Three whole specimens, one of them (MNHN-F A22689-L18452) from Cucuron, another (MNHN-F A22691 L18454) from S. Restitute (Drôme), the third (MNHN-F A22690-L18453) from S. Paul Trois Châteaux.

DESCRIPTION. Very small-sized species (TL=20-38 mm). Test a little wider than long (mean TW=110% TL), with the anterior part restricted and rounded posteriorly. Test low (mean TH=10% TL). Margin rather thin with shallow sinuities in correspondence of the anterior ambulacra. Internal structure: central hollow sub-pentagonal. From the rear wall to the front, the length of the hollow roughly corresponds to PL. Radial cavity: as in the diagnosis of the genus. Internal buttress system very reduced around the central hollow, the peripheral ballast system is densely packed above all close to the test margin. Apical system slightly anteriorly eccentric (L4=56% TL) and middle-sized (8.4% TL). Petalodium rather

small (mean PL= 46.5% TL). Posterior petals shorter than the frontal one (mean L5=24%TL, L9=20% TL); the width of the petals is comparable: L6=12% TL, L10=12% TL. Interporiferous zone 1.5 times larger than a poriferous one. Petals likely open distally. Tips of the posterior petals rather far from the corresponding lunules (mean L3= 9% TL), the space is occupied by two or three plates in each column. Basicoronal circlet large (L13=15.9% TL). The length of plate 1 in the oral interambulacral 5 is about 10% TL. In the oral interambulacrum 5, columns “a” and “b” are made of only two post-basicoronal plates; plate 5.a.2 has a very broad base and is paired to 5.b.2 but remains in touch with the two post-basicoronal plates of the ambulacra I and V. As a whole, there are only 10 plates per column in the interambulacrum 5. Lunules middle-sized (mean WI=140), sub-circular to radially elongate ovoidal (range SI=1.09–1.34); they may be different in the same specimen. They are separated from the respective petal tips by three plates per column. Peristome large (ϕ ps=5.2% TL), opening subcentrally. Periproct middle-sized (ϕ pc=2.3% TL), not too far from the posterior margin (L11=8.2% TL); it opens close to the conjunction of plates 5.a.2–5.a.3–5.b.2–5.b.3.

DISTRIBUTION. Langhian-Serravallian of Blanqui, near Cucuron (Vaucluse - France). Saint-Paul-Trois-Châteaux and S. Restitute, may be also Cadenet, near Vaugines (Drome - France). After Lambert (1907) also “Helvétien” of St-Chrystol, near Nissan (Hérault).

REMARKS. *Paraamphiope baquiei* is distinguished mainly by its very small size (mean TL=30 mm), almost rounded lunules and in having only 10 plates on the whole in the interambulacrum 5, the lowest number so far known for an astryclipeid. *Paraamphiope agassizi* differs also by the presence of three to five plates in each column between the tips of the posterior petals and the respective lunules.

Genus *Sculpsitechinus* Stara et Sanciu (2014)

TYPE SPECIES. *Sculpsitechinus auritus* (Leske, 1778) = *Echinodiscus auritus* (Leske, 1778).

TYPE MATERIAL. The neotype (Stara & Sanciu, 2014), represented by a well preserved Recent specimen (MZE.CA.MAC.IVM109) from Tulear, Madagascar.

DIAGNOSIS. Partially emended from Stara & Sanciu (2014):

- Test flat with thin margin. Oral face flat to slightly concave.
- Internal visceral hollow almost as wide as the petalodium, sub-circular to polygonal in shape, with walls reinforced by a network of thin trabeculae.
- Apical disc monobasal, subcentral or slightly anterior to centre, with four small gonopores.
- Interambulacra a little wider than ambulacra at the ambitus.
- All five petals similar in length; the anterior one sometimes longer. Petalodium generally small, ranging from PL=30 to 48% TL. In a Recent Iranian population, it reaches 60% TL (Fatemi et al., 2016). Petals well developed and often open distally; angle β = 48–70°.
- Three to four post-basicoronal plates per column in interambulacrum 5, with the first two normally partially paired (or slightly staggered) and normally with only the plate 5.b.2 in contact with the adjacent ambulacral plates.
- Width of the interambulacrum 5 at the margin about 30–38% TL.
- Basicoronal circlet pentastellate with interambulacral plates sometime elongated distally and commonly separated from the post-basicoronal ones.
- Two radially elongate ellipsoidal lunules or slit-like notches present in the posterior ambulacra; two to five couples of plates separate the tip of the petals from the corresponding lunules.
- Peristome small, subcentral or slightly anteriorly located.
- Periproct circular, small, rather far from the posterior margin (L11>12% TL; mostly 13–26% TL).
- Main food grooves strongly branched; several short and fine secondary ramifications branch off distally and also along their middle part. Tube-feet extending into the interambulacral zones.
- Tuberculation dense, made of very small, perforate and crenulate tubercles, which are larger on the oral side.

DISTRIBUTION. Early Miocene of France, Middle

Miocene of Papua New Guinea and Pliocene of Red Sea (Egypt) and Khark Island, Persian Gulf (Iran). Recent: Red Sea, Persian Gulf, Indian Ocean, Australia, Indonesian Archipelago and West-Pacific.

Species included:

- *Sculpsitechinus auritus* (L. Agassiz, 1838), Recent; Madagascar, Red Sea, Indian Ocean and West Pacific.
- *Sculpsitechinus tenuissimus* (L. Agassiz, 1847), Recent; Indonesia, New Caledonia, Papua New Guinea and Palau, Micronesia.
- *Sculpsitechinus boulei* (Cottreau, 1914), Early Miocene, France.
- *Sculpsitechinus* sp. 1 Stara & Sanci (2014), Recent, Philippines.
- *Sculpsitechinus* sp. 2 Stara & Sanci (2014), Middle Miocene, Papua New Guinea.
- *Sculpsitechinus iraniensis* Fatemi, Attaran-Fariman et Stara, 2016, Recent; Chabahar Bay, Persian Gulf.

Species previously assigned to *Amphiope* herein transferred to the genus *Sculpsitechinus*:

***Sculpsitechinus boulei* (Cottreau, 1914)**

Figs. 180–184; Figs. 79–81

1914. *Amphiope boulei* nov. sp. Cottreau: pp. 92–94; figs. 17–18; pl. 5, fig. 9

1915. *Amphiope boulei* Cottreau - Lambert: p. 219

1988. *Amphiope boulei* Cottreau - Philippe: pp. 167–169; pl. 16, fig. 9.

TYPE LOCALITY. Carry-le-Rouet (Bouche du Rhône - France). Aquitanian.

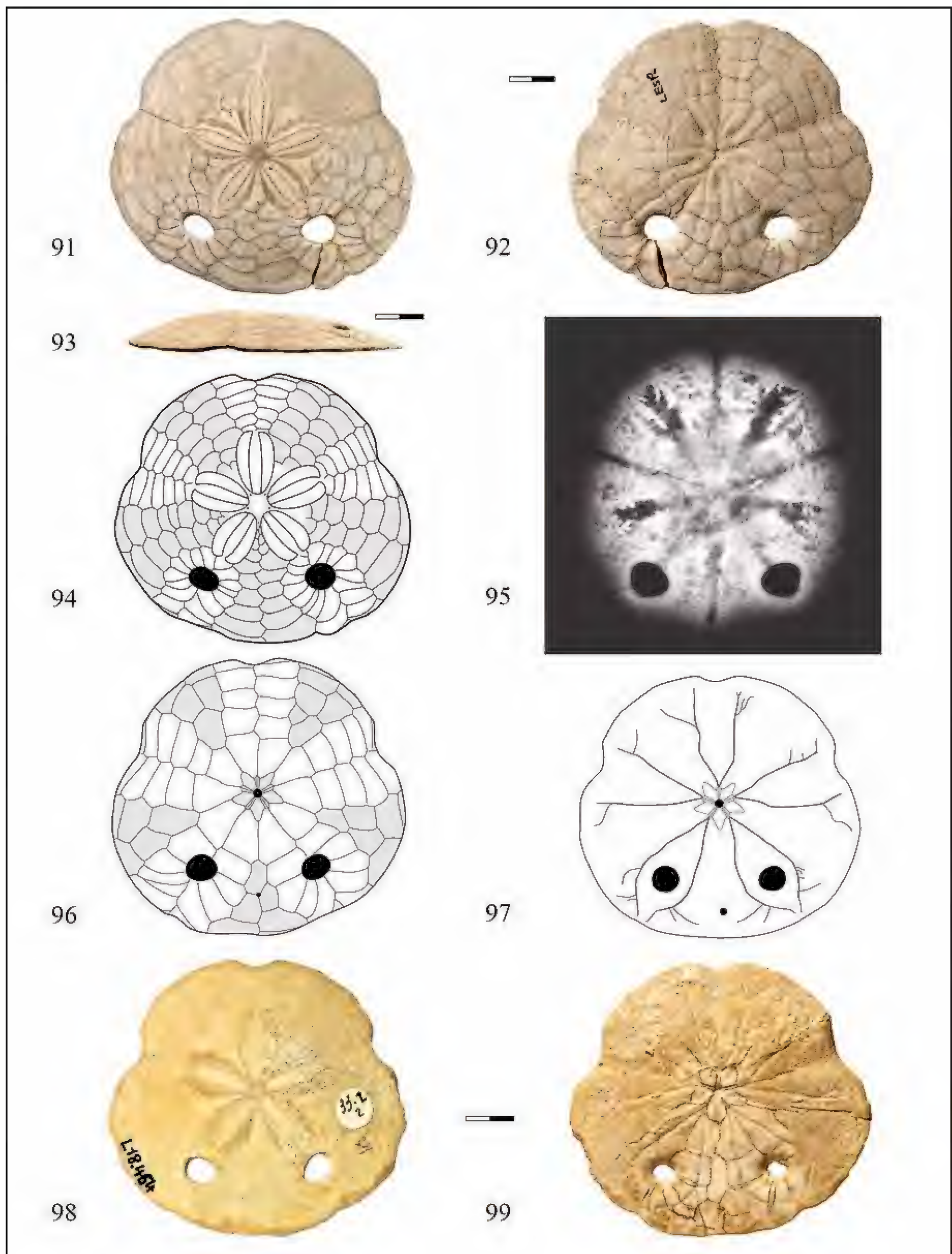
TYPE MATERIAL. Holotype (MNHN-F R62136).

DESCRIPTION. Medium sized test (TL \approx 95 mm), slightly longer than large (TW=97% TL). Outline likely roundish, with the anterior part slightly restricted. The test is very low (TH=8% TL), with highest point anterior to the apical disc. Oral face flat, with interambulacral areas slightly inflated and ambulacral areas with a shallow and broad depression deepening towards the peristome. Margin rather thin with shallow notches in correspondence of the anterior paired ambulacra. Internal structure: central hollow sub-circular and

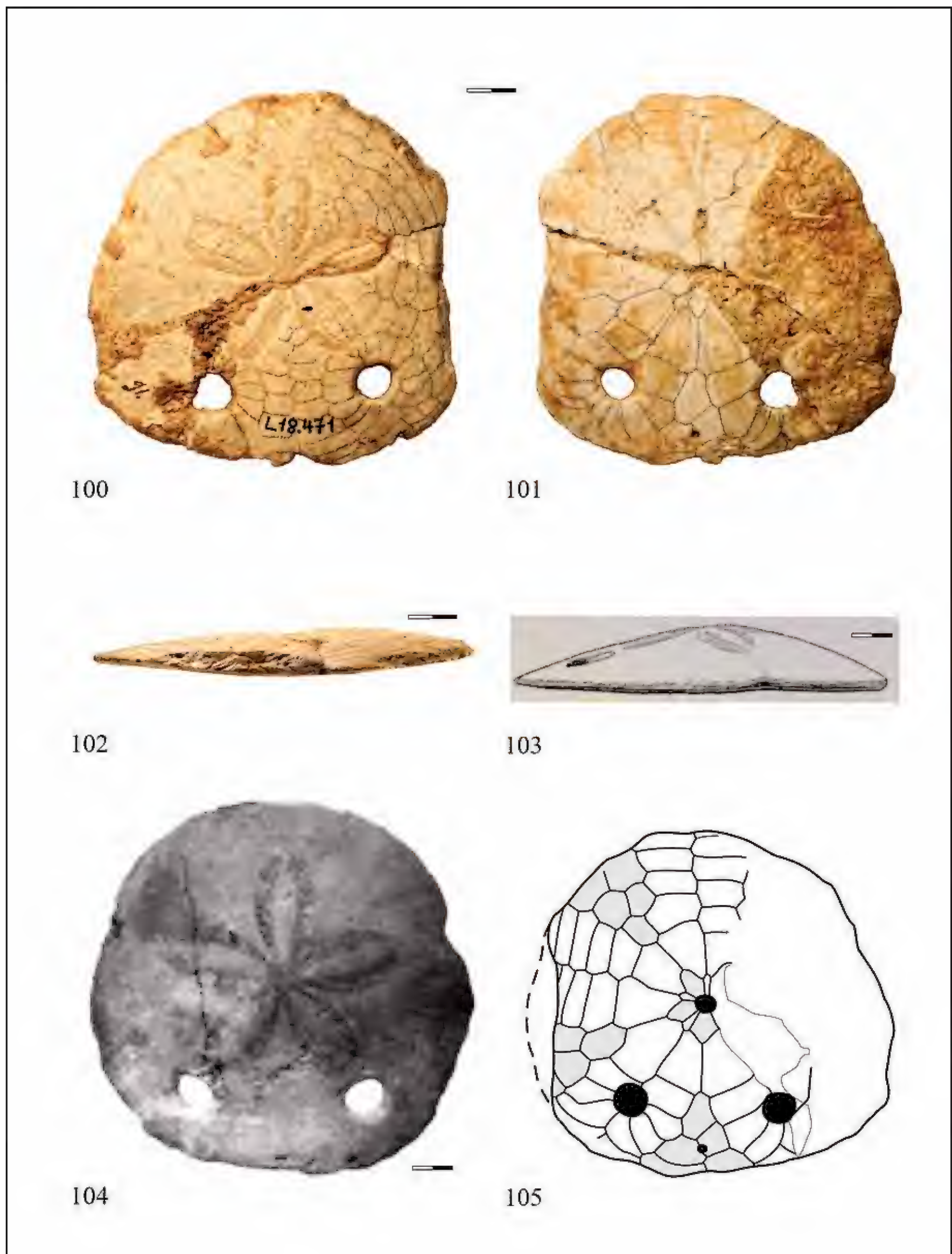
very small (Fig. 180). Ten cavities branch from the central hollow along the ambulacra and the interambulacra; the longest one extends along the interambulacrum 5 and leads to the periproct. The internal structure is reduced anteriorly and becomes denser only towards the posterior test margin. Apical system slightly anteriorly eccentric (L4 about 56% TL) and medium-sized. Petalodium small (PL=47% TL). Posterior petals shorter than the frontal one (L5=23% TL, L9= 20% TL), their width is proportionally almost comparable (L6 and L10=10% TL). In the posterior petals the width of the interporiferous and poriferous zones is 2.9% and 3.9% TL, respectively. In the frontal ambulacrum, the width of an interporiferous zone is 1.3 times that of the poriferous one. Petals likely open distally. In the oral interambulacrum 5, column “b” is made of three post-basicoronal plates column “a” of two and a small part of the third (Fig. 81). Plate 5.a.2 is almost paired to 5.b.2 but remains in touch with the two post-basicoronal plates of the ambulacra I and V. As a whole, there are 12–13 plates in each column of the interambulacrum 5. Lunules small (WI=71), radially elongate, ovoidal (SI=1,6). The tips of the posterior petals are rather far from the corresponding lunules (L3=11% TL) and separated by four plates per column. Peristome middle-sized (ϕ ps=4% TL), opening centrally. Periproct wide (ϕ pc=4% TL), rather far from the posterior margin (L11= about 12% TL) and opening at the conjunction of plates 5.a.2–5.b.2–5.b.3. Food grooves very branched distally; however, only a part of the secondary branches are visible due to the bad preservation of the oral surface.

DISTRIBUTION. Aquitanian of Carry-le-Rouet (Bouche du Rhône - France).

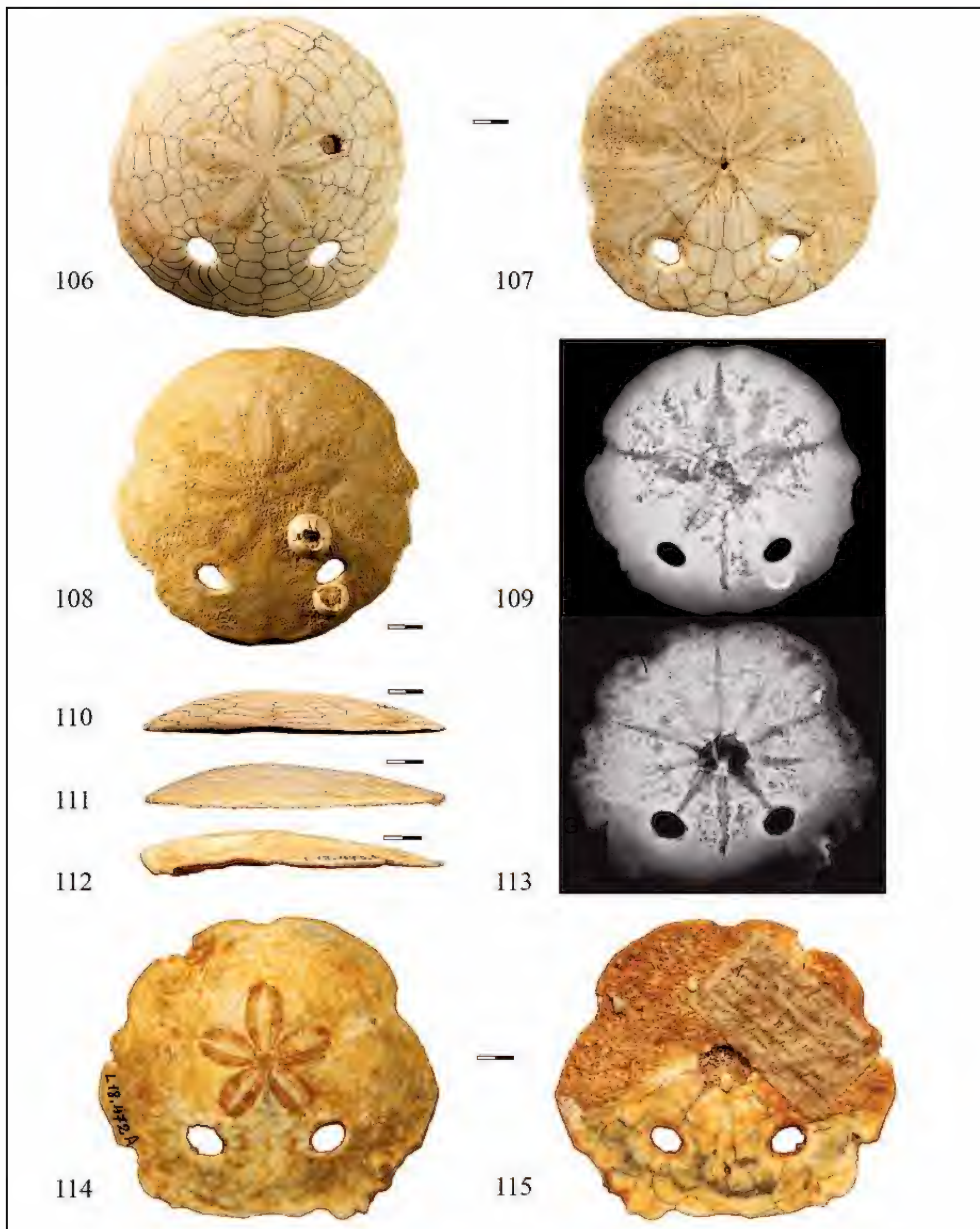
REMARKS. *Sculpsitechinus boulei* is distinguished from *S. auritus*, *S. iraniensis* and *Sculpsitechinus* sp.1 in having elliptical lunules, instead of open notches. It differs from *S. tenuissimus* by its smaller lunules, which are not elongate and closer to the posterior margin, by much large petalodium (mean PL=47% TL against 30%). *Sculpsitechinus boulei* is apparently close to *Sculpsitechinus* sp. 2, however they are here maintained separate, since the plate-patterns are unknown and they likely lived in different climatic and paleoecological conditions.



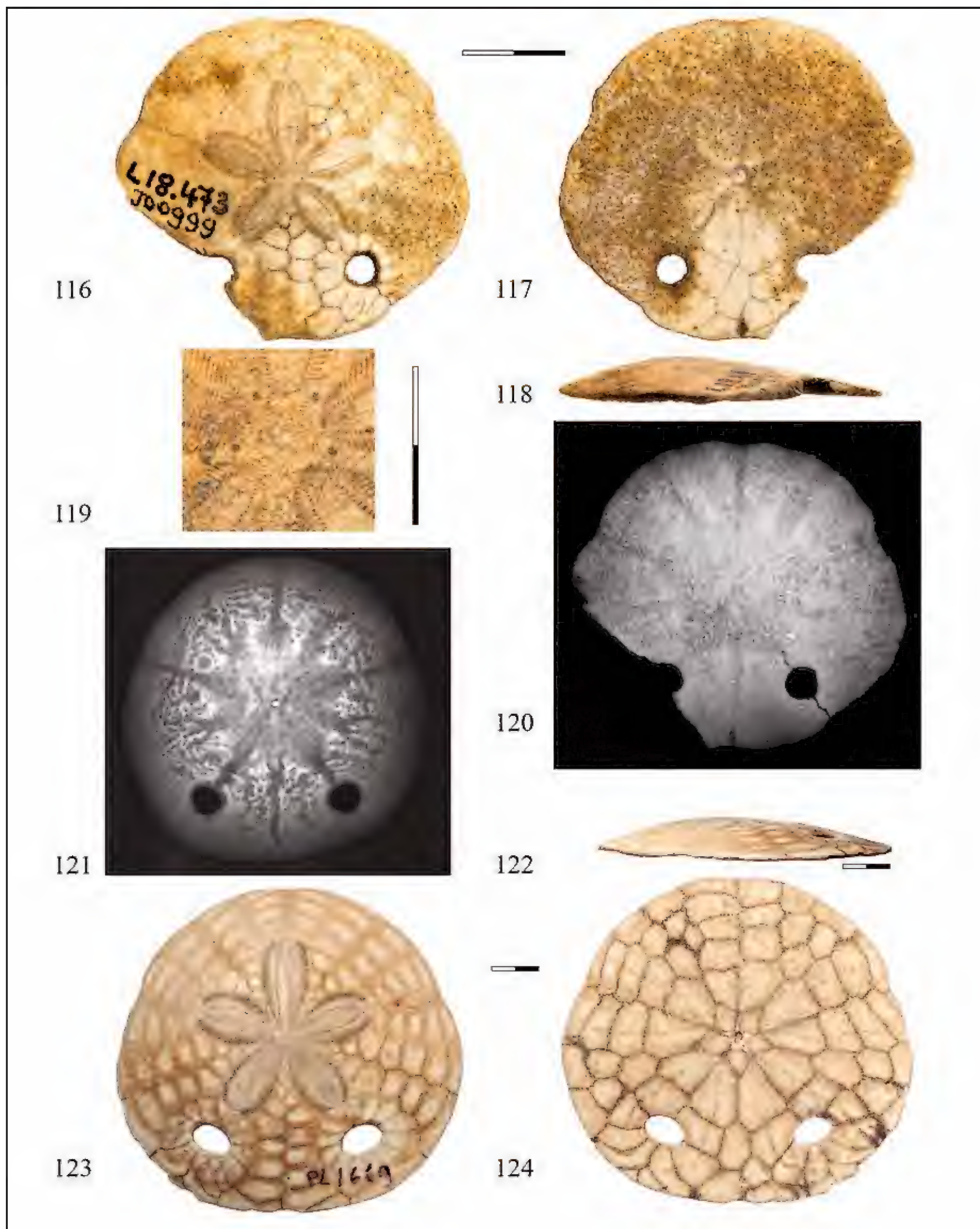
Figures 91–99. *Amphiope bioculata*. Aboral (Fig. 91), oral (Fig. 92) and lateral (Fig. 93) views of the specimen that will be proposed as neotype (MNHN-F.A 57777), from Lespignan. Fig. 94, 96: scheme of aboral and oral faces. Fig. 95, 97: radio-graph and food grooves scheme, respectively of the proposed neotype (MNHN-F.A 57777). Figs. 98, 99: aboral and oral view of a specimen (MNHN-F.A22701.L18464e) from Nissan les Tuilières.



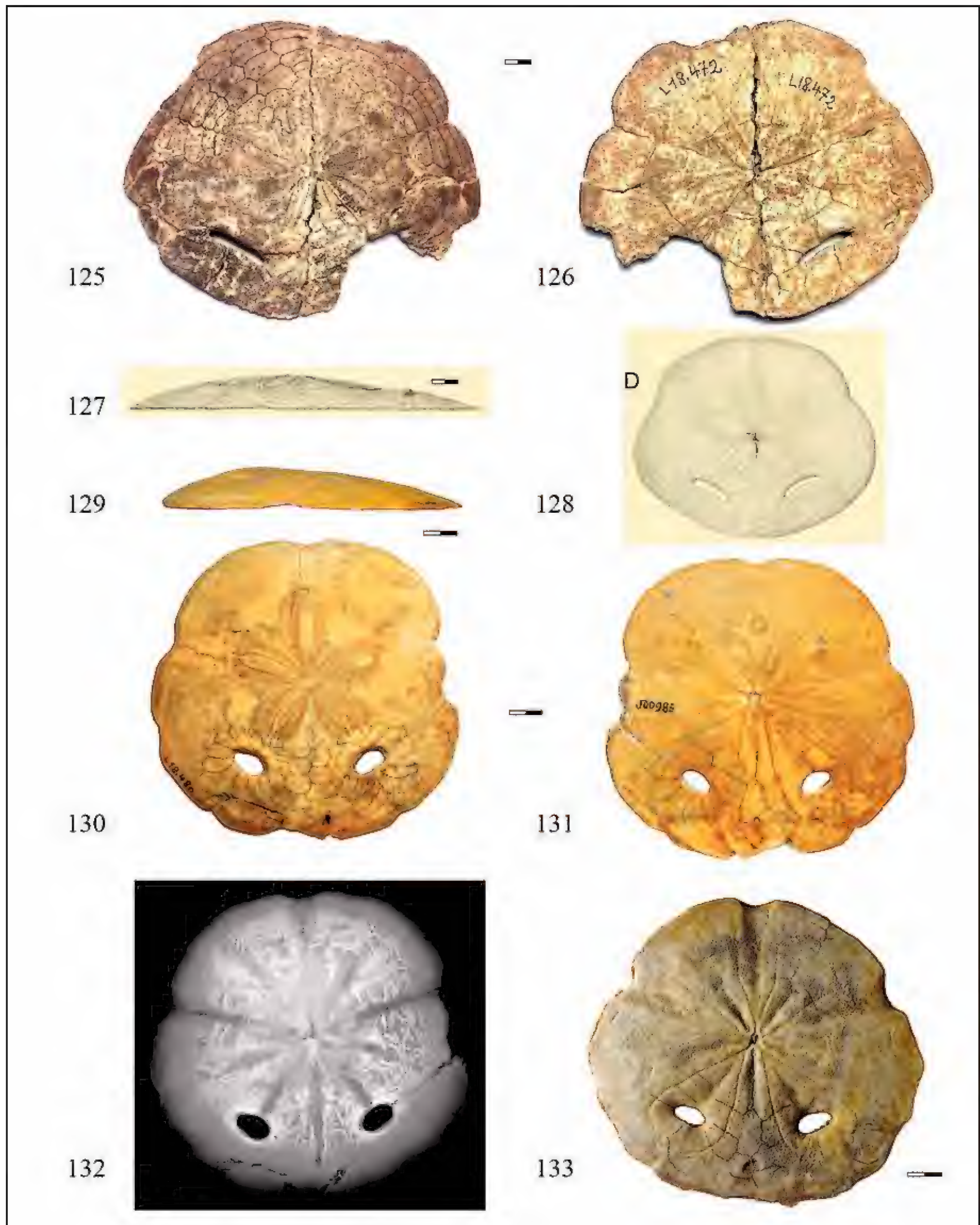
Figures 100–105. *Amphiope elliptica*. Aboral (Fig. 100), oral (Fig. 101), lateral (Fig. 102) views and (Fig. 105) adoral scheme of specimen (MNHN-F.A22706-L18.471) from Carry-le-Rouet (Bouche du Rhône, France); Mold T9 of the type: lateral drawing (Fig. 103) and aboral view (Fig. 104), from Cottreau (1914).



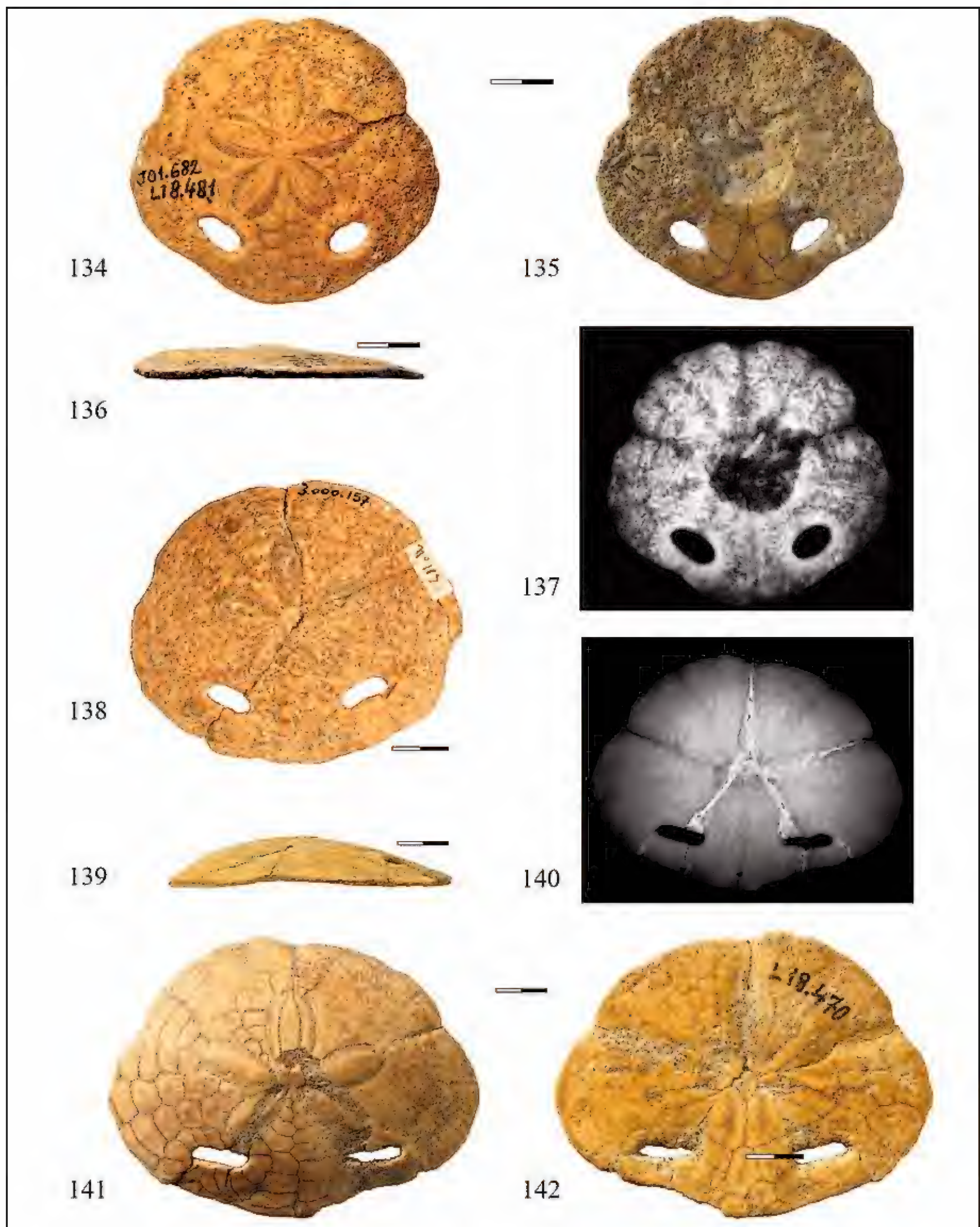
Figures 106–115. *Amphiope ovalifera* (Figs. 106–111) and *A. lorioli* (Figs. 112–115). Figs. 106, 107, 110: *A. ovalifera*, aboral, oral and lateral views (MNHN-F. A22710 L18.477c), topo-typic specimen from Gornac (France): the test surface has been artificially abraded to highlight the plating structure. Figs. 108, 109: specimen MNHN-F. A22710 L18.477l, from Gornac, Fig. 108: aboral view, Fig. 109: radiography showing the high density of the internal support system towards the margin in this species; Fig. 111: lateral view. Figs. 112–115: *A. lorioli*, topotypic specimen from St-Félix-de-Lodez (Hérault - France). Specimen (MNHN-F A22707-L 18472Aa): lateral (Fig. 112), aboral (Fig. 113) and oral (Fig. 114) views; radiography (Fig. 115) showing the high density of the internal support system only towards the margin in this species.



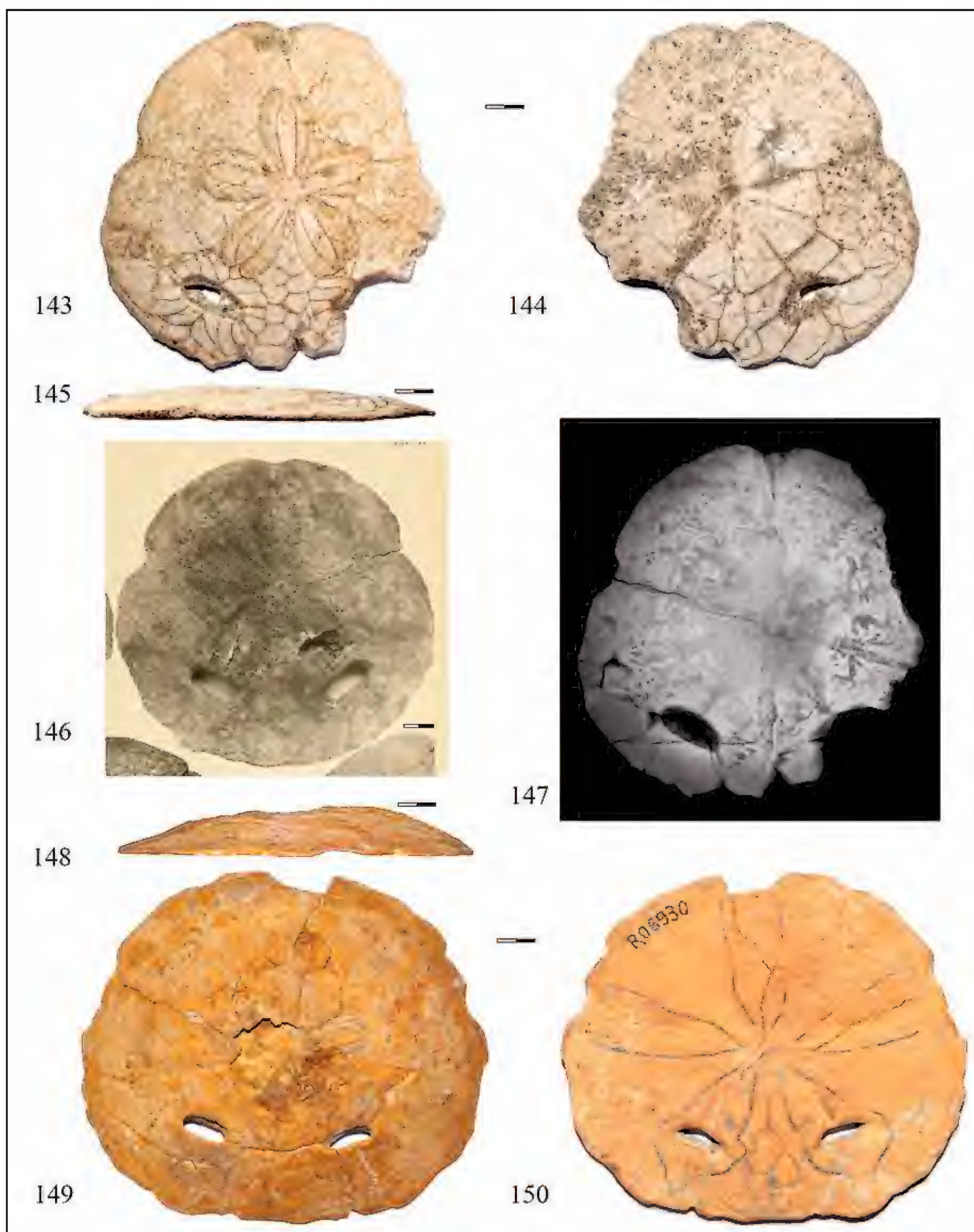
Figures 116–124. *Amphiope ludovici* (Figs. 116–120) and *A. romani* n. sp. (Figs. 121–124). *Amphiope ludovici*: syntype (MNHN-F.J00999 L18473) from Blanqui (Vaucluse - France). Aboral (Fig. 116), oral (Fig. 117) and lateral (Fig. 118) views; magnification of the apical system (Fig. 119) highlighting the presence of 4 gonopores in adult individuals, radiography (Fig. 120). *Amphiope romani* n. sp., holotype (MNHN-F.A57780.PL1669) from Channay-sur-Lathan, Touraine (France): radiography (Fig. 121) and lateral (Fig. 122), aboral (Fig. 123) and oral (Fig. 124) views; the test surface has been partially abraded to highlight the sutures between the plates.



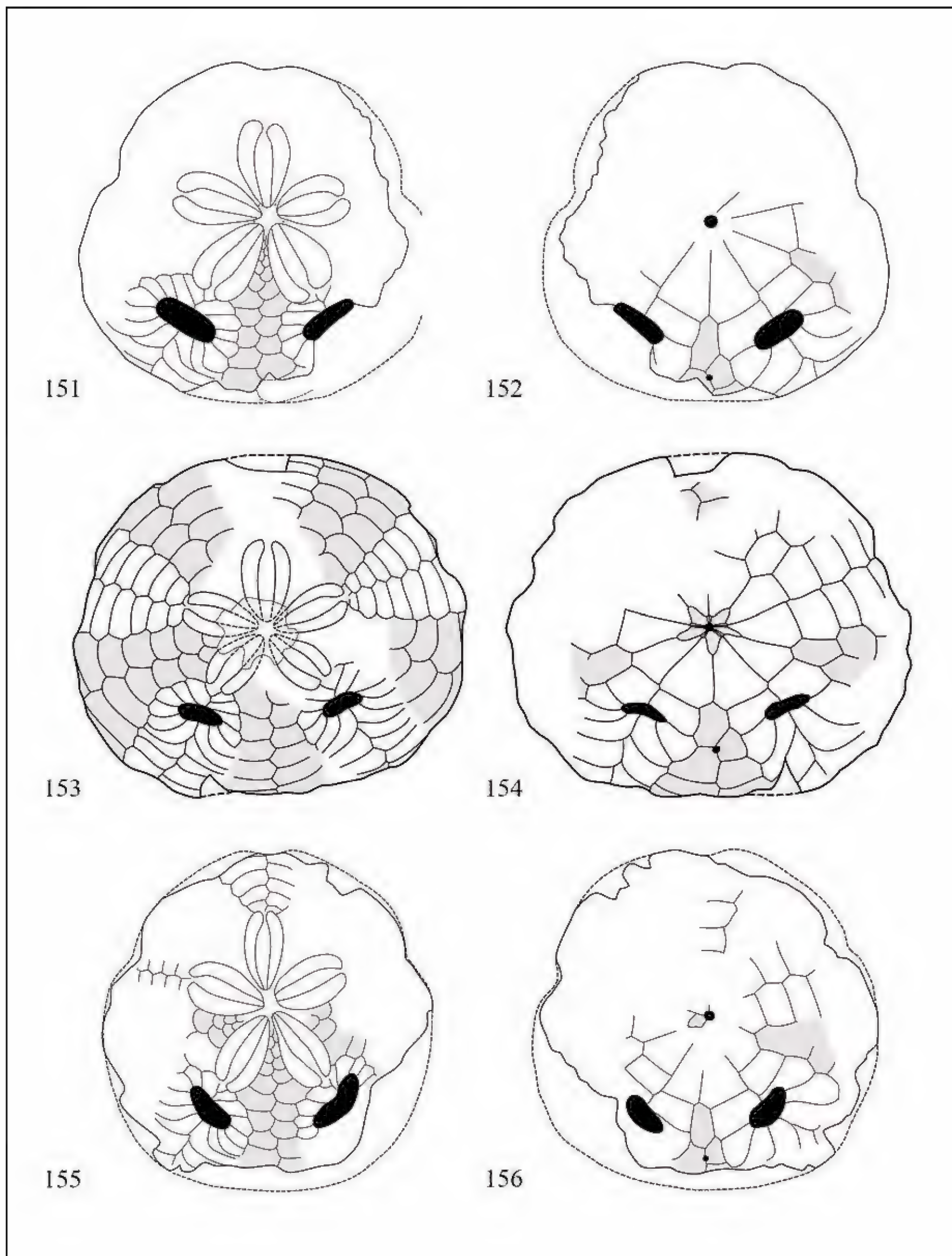
Figures 125–133. *Amphiope hollandei* (Figs. 125–128) and *A. sarasini* (Figs. 129–133). *Amphiope hollandei*, holotype from Bonifacio (Corsica, France): aboral (Fig. 125) and oral (Fig. 126) views; subjective reconstruction (Figs. 127, 128) reported in Cotteau (1877: pl. 9, figs. 6, 7). *Amphiope sarasini*, syntype (MNHN-F J00985.L18480) from Cruzy: lateral (Fig. 129), aboral (Fig. 130) and oral (Fig. 131) views and radiography (Fig. 132); oral face view (Fig. 133), taken with grazing light to highlight depressions and food grooves in specimen MNHN-F.A.57789 from Cruzy.



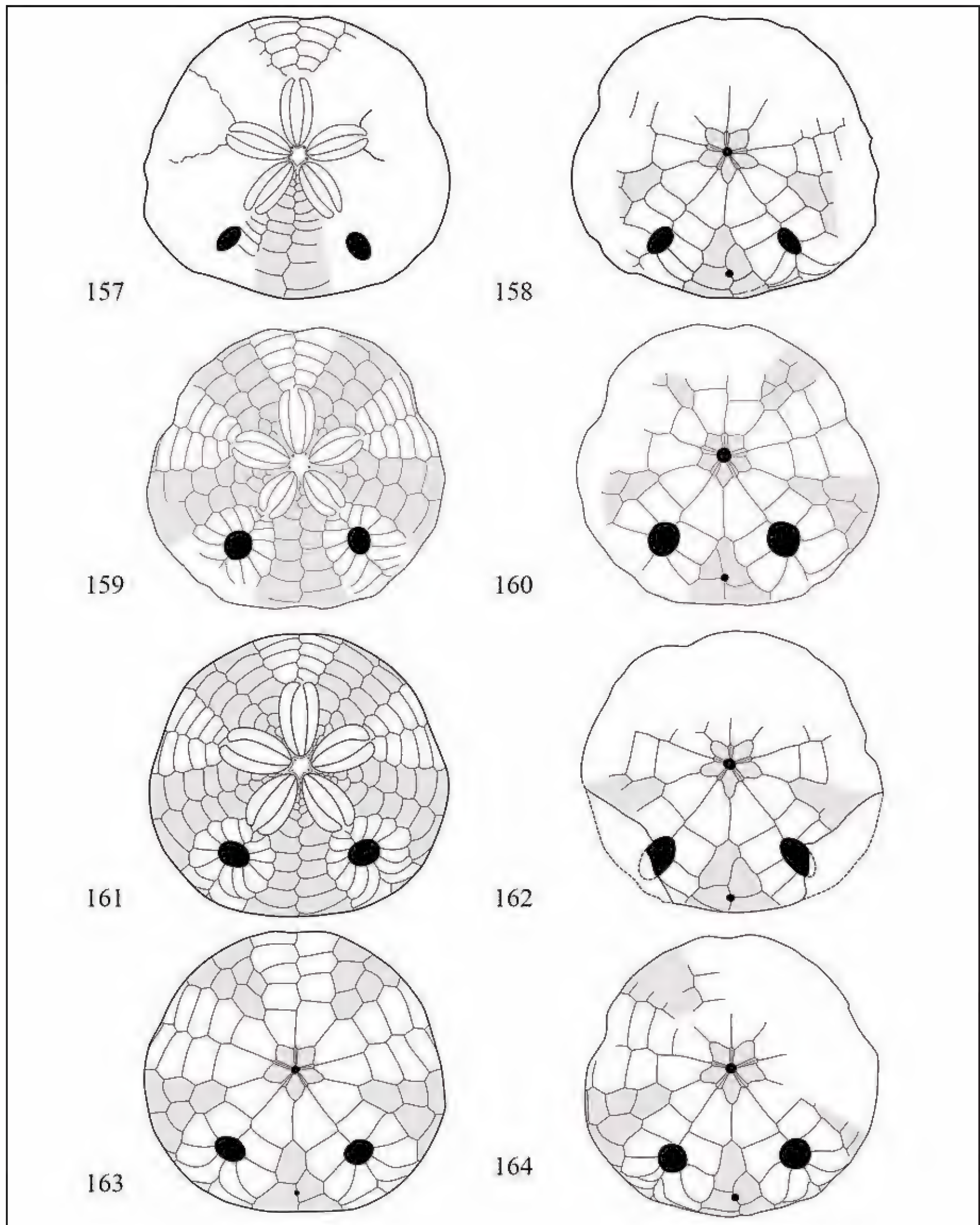
Figures 134–142. *Amphiope transversifora* (Figs. 134–137) and *A. deyrieri* (Figs. 138–142). *Amphiope transversifora*, syntype (MNHN-F J01682): aboral (Fig. 134), oral (Fig. 135) and lateral (Fig. 136) views; radiography (Fig. 137). *Amphiope deyrieri* holotype (MRA3.000.157 (117), from Vaugines, aboral view (Fig. 138); specimen MNHN-F.A22705.L18.470b from Cadenet, Vaugines (Vaucluse - France): lateral (Fig. 139), aboral (Fig. 141), oral (Fig. 142) and radiography (Fig. 140).



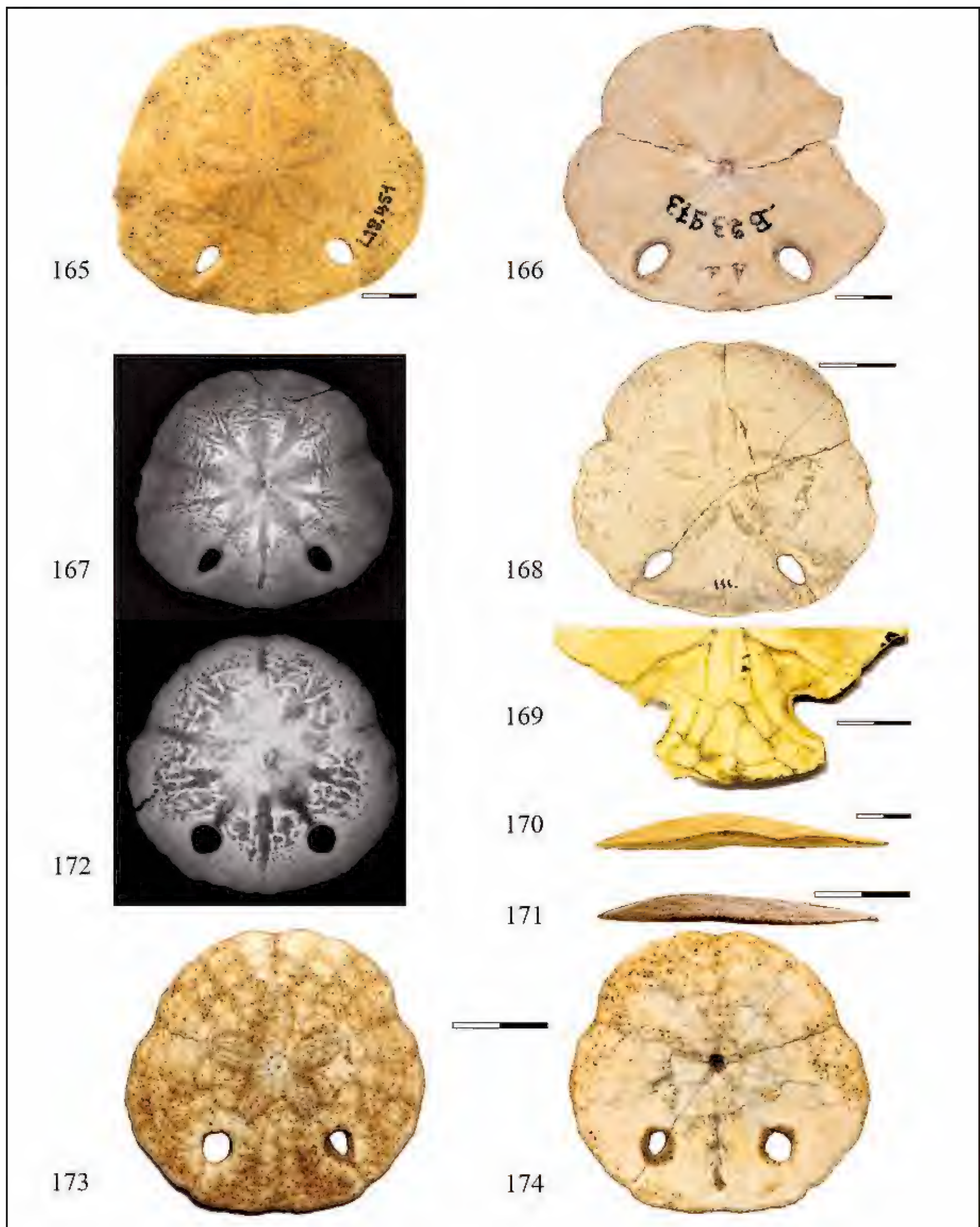
Figures 143–150. *Amphiope pallavicinoi* (Figs. 143–147) and *A. tipasensis* (Figs. 148–150). *Amphiope pallavicinoi*, neotype MDLCA 23583 from Bessude (Italy): aboral (Fig. 143), adoral (Fig. 144) and lateral (Fig. 145) views, original illustration (Lovisato, 1914) of a specimen from Torralba (Fig. 146) and radiography (Fig. 147). *Amphiope tipasensis*, holotype (MNHN-F.R06930), from Tipasa province (Algeria): lateral (Fig. 148), aboral (Fig. 149) and adoral (Fig. 150) views.



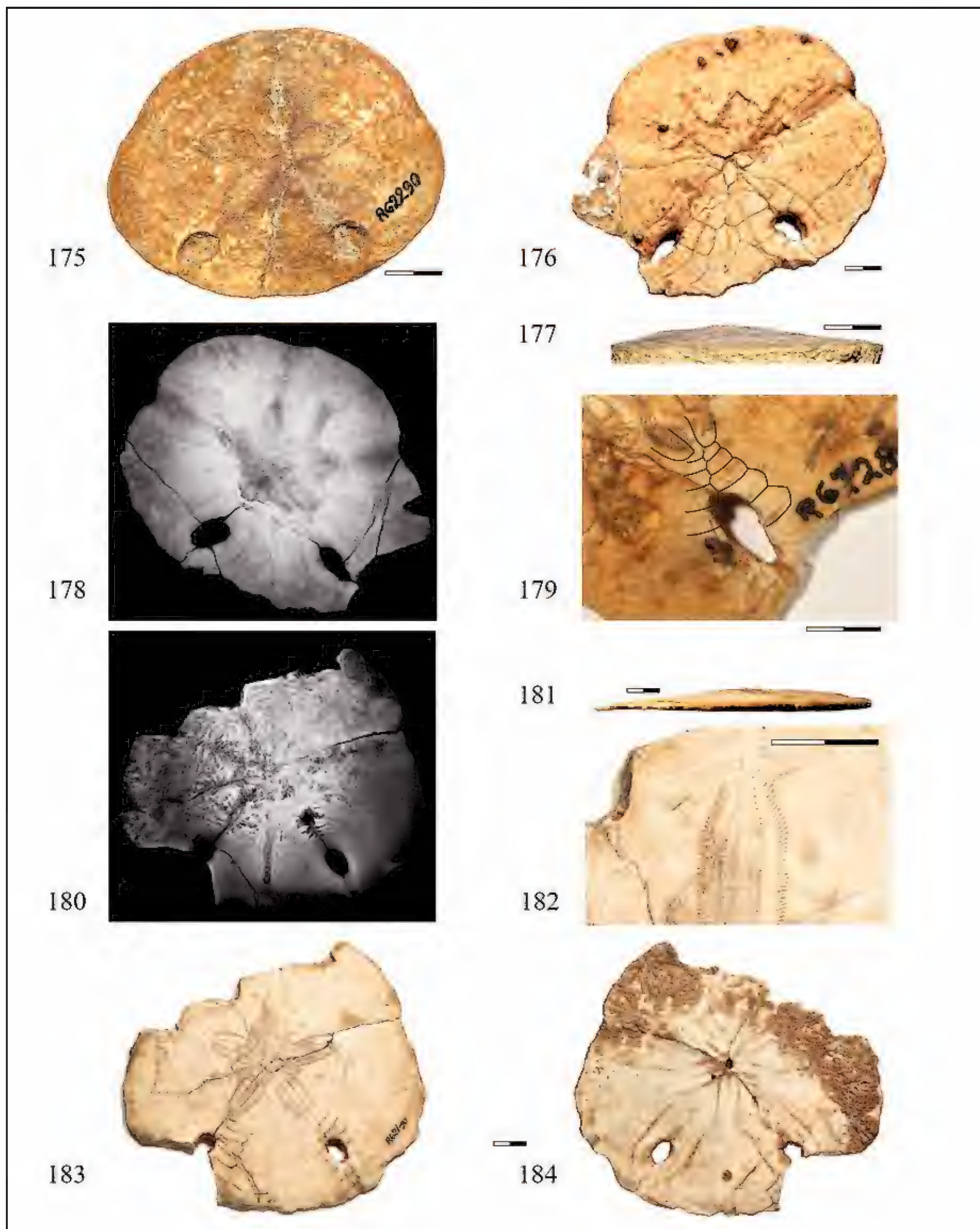
Figures 151–156. Aboral and oral plating schemes of *Amphiope pallavicinoi* (MDLCA 23583) (Figs. 151, 152), *A. tipasensis* (MNHN-F.R06930) (Figs. 153, 154) and *A. palpebrata* (MNHN-F. L18.478) (Figs. 155, 156).



Figures 157–164. Oral and/or aboral plating schemes of two species belonging to *Amphiope* and two belonging to *Paraamphiope*. Figs. 157, 158: *Paraamphiope agassizi* (MNHN-F.A22688), from Montségur. Figs. 159, 160: *P. baquiei* (MNHN-F A22689-L18452), from Cucuron. Figs. 161, 163: *A. romani* n. sp. (MNHN-F.A57780.PL1669), from Channay-sur-Lathan. Fig. 162: *P. agassizi* (MNHN-F. A22687), from Pellegrue. Fig. 164: *A. romani turonensis* (MNHN-F.A22713. L.18482a), from Oisly.



Figures 165–174. *Paraamphiope agassizi* (Figs. 165–170) and *P. baquiei* (Figs. 171–174). *Paraamphiope agassizi*, specimen MNHN-F.A22688.L18.451, from Montségur: aboral (Fig. 165) and lateral (Fig. 170) views and radiography (Fig. 167); syntype (MNHN-F. B23973) from Montségur (Gironde), oral view (Fig. 166); syntype (MHNbX 111 6.194) from S. Albert (Gironde) (Fig. 168); specimen MNHN-F.A22687.L18.450-sn2, from Pellegrue: oral view (Fig. 169) highlighting the almost paired first postbasicoronal plates in the interambulacrum 5. *Paraamphiope baquiei*, specimen MNHN-F.A22689.L18452, from Cucuron (France): lateral (Fig. 171), radiography (Fig. 172), aboral (Fig. 173) and oral (Fig. 174) views.



Figures 175–184. *Paraamphiope cherichirensis* (Figs. 175–179) and *Sculpsitechinus boulei* (Figs. 180–184). *Paraamphiope cherichirensis*, holotype (MNHN-F.R62290), from Djebel Cherichira (Tunisia): aboral (Fig. 175) and lateral (Fig. 177) views; specimen (MNHN-F.R62288) from Bou Golrine (Tunisia): oral view (Fig. 176), radiography (Fig. 178) and close up (Fig. 179) of the aboral face showing the plates between the petal tip and the lunule. *Sculpsitechinus boulei*, holotype (MNHN-F R62136), from Carry le Rouet (France): radiography (Fig. 180), lateral view (Fig. 181) and close-up of the left anterior paired front petal (Fig. 182); aboral (Fig. 183) and oral (Fig. 184) views.

Species	New arrangement / validity	Type locality	Stratigraphy	Type material
<i>A. bioculata</i> (Des Moulins, 1837)	valid	Sure, près Bollène (unknown locality)	unknown	sintype (MNHBX 2014.6.189)
		Lespignan (Hérault - France)	Langhian-Serravallian	neotype (MNHN-F-A57777)
<i>A. perspicillata</i> Agassiz, 1841	<i>species inquirenda</i>	Rennes (France)	unknown	wanting - repository unknown
<i>A. elliptica</i> Desor, 1847	valid	Carry Le Rouet (Bouche du Rhône - France)	late Aquitanian- early Burdigalian	holotype wanting
<i>A. agassizi</i> Des Moulins in Cotteau, 1865	recombined into <i>Paraamphiope agassizi</i>	La Réole and Montségur (Gironde - France)	Rupelian-Chatian (Oligocene)	n. 2 syntypes (MNHBX 111.6-194, MNHN B23973)
<i>A. ovalifera</i> (Des Moulins in Benoist, 1875)	valid	Gornac (Gironde - France)	early Aquitanian	n. 2 syntypes (MNHBx 2014.6.180.2)
<i>A. hollandei</i> Cotteau, 1877	valid	Bonifacio (Corsica - France)	Burdigalian	holotype (MNHN-F A22378. L18472)
<i>A. arcuata</i> Fuchs, 1882	recombined into <i>Paraamphiope arcuata</i>	Oasis Siouah (Egypt)	Middle Miocene	holotype illustrated, but its repository is unknown
<i>A. truncata</i> Fuchs, 1882	not belonging to <i>Amphiope</i> , not discussed here	Oasis Siouah (Egypt)	Middle Miocene	?
<i>A. styriaca</i> Hoernes, 1883	<i>nomen dubium</i>	Leibnitz (Austria)	Langhian	holotype (UGP 1880.XX.5)
<i>A. palpebrata</i> Pomel, 1887	valid	Algeria	Burdigalian	only a topo-typic specimen (MNHN. L18.478)
<i>A. depressa</i> Pomel, 1887	valid	Algeria	"Helvétien"	repository unknown
<i>A. personata</i> Pomel, 1887	<i>species inquirenda</i>	Algeria	Burdigalian	repository unknown; type locality untraceable
<i>A. villei</i> Pomel, 1887	<i>species inquirenda</i>	Algeria	Burdigalian	repository unknown; type locality untraceable
<i>A. fuchsi</i> Fourtau, 1899	junior synonym of <i>A. truncata</i> not belonging to <i>Amphiope</i> not discussed here	unknown locality (Egypt)	Middle Miocene	?
<i>A. cherichirensis</i> Thomas & Gauthier, 1889	recombined into <i>Paraamphiope cherichirensis</i>	Djebel Cherichira (Tunisia)	Burdigalian	holotype (MNHN-F-R62290)
<i>A. dessii</i> Lovisato in Cotteau, 1895	<i>species inquirenda</i>	Nurri (Sardinia - Italy)	Burdigalian	type series lost in 1943 type locality unknown
<i>A. lovisatoi</i> Cotteau, 1895	valid	Chiaromonti (Sardinia)	late Burdigalian	neotype (MAC.PL1706)
<i>A. koehleri</i> Mortensen, 1905	not discussed here	?	?	?
<i>A. neuparthi</i> De Loriol, 1905	valid	Angola	Burdigalian	sintype (LNEG)
<i>A. baquiei</i> Lambert, 1907	recombined into <i>Paraamphiope baquiei</i>	Cucuron (Vaucluse - France)	Langhian-Tortonian	neotype (MRA 3.000.154)
<i>A. lorioli</i> Lambert, 1907	valid	St-Félix-de-Lodez (Hérault - France)	Langhian-Tortonian	(MNHN-F A22707-L 18472A)

Table 1/1. Summary list of the Astrilypeidae taxa examined in this work. Data concerning the taxonomic arrangement, type locality/type area, stratigraphy and repository of the type material have been updated, for each taxon, when possible.

Species	New arrangement / validity	Type locality	Stratigraphy	Type material
<i>A. duffi</i> Gregory, 1911	not belonging to <i>Amphiope</i> not discussed here	Cirenaica (Libya)	Oligocene	Two syntypes: (NHMUK CY66/E11350, Cy264/E11349)
<i>A. montezemoloi</i> Lovisato, 1911	valid	S. Giorgio (Sardinia - Italy)	Burdigalian-early Langhian	neotype (MAC.PL1827)
<i>A. sarasini</i> Lambert, 1912	valid	Cruzy (Hérault - France)	Tortonian	syntype (MNHN-F J00985)
<i>A. transversifora</i> Lambert, 1912	valid	Saint-Paul-Trois-Châteaux (France)	Langhian	syntype (MNHN-F J01682)
<i>A. deyrieri</i> Lambert, 1912	valid	Vaugines (Vaucluse - France)	Langhian-Serravallian	holotype (MRA 3.000.157)
<i>A. laubei</i> Lambert, 1912	<i>nomen dubium</i> *	Niederkreuzstetten (Austria)	late Burdigalian	holotype (NHMW 1849.XX.39)
<i>A. pallavicinoides</i> Lovisato, 1914	valid	Ploaghe, the type-stratum crops out also at Bessude (Sardinia - Italy)	late Burdigalian-Langhian ?	type series lost - neotype MDLCA 23583
<i>A. calvii</i> Lovisato, 1914	<i>species inquirenda</i>	Torralba (Sardinia - Italy)	late Burdigalian-Langhian ?	type series lost type locality untraceable
<i>A. boulei</i> Cottreau, 1914	recombined into <i>Sculpsitechinus boulei</i>	Carry-le-Rouet (Bouches du Rhône - France)	Aquitanian	holotype (MNHN-F R62136)
<i>A. ludovici</i> Lambert, 1915	valid	Cucuron (Vaucluse - France)	Tortonian	n.2 syntypes (MNHN J00999 L18473, L18474)
<i>A. bioculata aequipetala</i> Lambert 1915	<i>subspecies inquirenda</i> .	Montréal (Gers - France)	Burdigalian-Serravallian ?	repository unknown
<i>A. doderleini</i> Lambert & Thiéry 1921	junior synonym of <i>Echinodiscus truncatus</i> (Agassiz L., 1841)	unknown	Recent	MNHN-F
<i>A. labriei</i> Lambert, 1927	<i>species inquirenda</i>	St-Félix-de-Lodez (Hérault - France)	unknown	repository unknown
<i>A. dallonii</i> Lambert, 1931	<i>species inquirenda</i>	Algeria	Middle Miocene	repository unknown
<i>A. bioculata philodonax</i> Lambert, 1927	<i>subspecies inquirenda</i>	Saucats (France)	Early Miocene	MHNBx 2014.6.189
<i>A. bioculata pelatensis</i> Fabre, 1933	<i>subspecies inquirenda</i>	France	Middle Miocene	repository unknown
<i>A. bioculata bentivegnae</i> Desio, 1934	<i>subspecies inquirenda</i>	Libya	Middle Miocene	repository unknown
<i>A. tipasensis</i> (Aymé & Roman, 1954)	valid	Tipasa (Algeria)	Pliocene	holotype (MNHN-F-R06930)

Table 1/2. Summary list of the Astrilypeidae taxa examined in this work. Data concerning the taxonomic arrangement, type locality/type area, stratigraphy and repository of the type material have been updated, for each taxon, when possible.

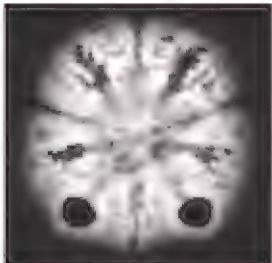

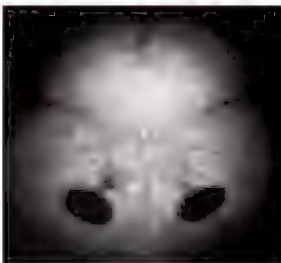
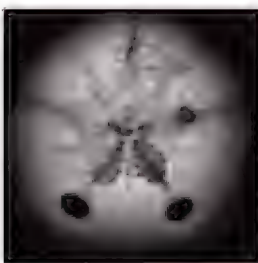

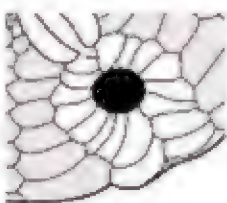




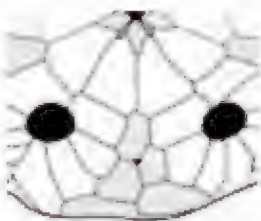
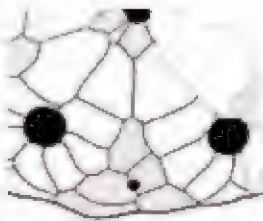



	<i>A. bioculata</i>	<i>A. elliptica</i>	<i>A. lovisatoi</i>	<i>A. ovalifera</i>	<i>A. lorioli</i>
TL (mm)	61	84.5	76	75	88
TH	16.5	17	10.1	13	11.7
TW	107	104	105.4	105	107
Internal structure (X-ray photographs)					
Shape and size of the central hollow	Subpentagonal-starring 50	Roundish -	Roundish to subpentagonal 50	Subpentagonal 46	Subpentagonal-starry 46
Internal test structure	Very light - large vacuoles also towards the margin	Light, denser towards the margin	Light very dense near the margin	Dense, almost massive towards the margin	Light
Apical disc position (L4)	57	56	62	55	58
Petalodium size (PL)	52	56	52	50	50
N (column a) N (column b)	2-3 3	2 3	2-3 3	2-3 3	2 3
N tot - interambulacrum 5	13-15	14-16	14-16	14-16	15-16
Length of plate 5.b.2	15.6 (27.8)	8 (17)	16 (29.5)	15.8 (25.5)	16 (29)
Lunules: WI SI	114 1.2	82 1.3	180 1.56	127 1.6	75 1.5
Lunules outline and distance from the posterior petals tips					
Periproct position (L11)	11.6	7	12	9.6	11.6
Oral scheme with the position of the periproct					
Remarks	Internal structure very light Plate 5.b.2 short	Basicoronal circlet large, L13=14% TL Plate 5.b.2 short	Margin sharp	Internal structure strong Basicoronal circlet very large (L13 up to 20% TL)	Lunules very small

Table 2/1. Comparison of the main morphological features of the species of *Amphiope* attributed to the informal *bioculata* group. If not otherwise indicated, the data report the mean value of the biometric measures expressed as % TL. N = number of post-basicoronal plates in the oral interambulacrum 5, N tot = whole number of plates in the interambulacrum 5. Red numbers = maximum values; blue numbers= minimum values.

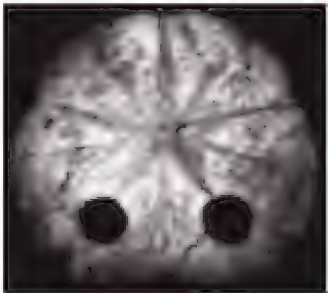
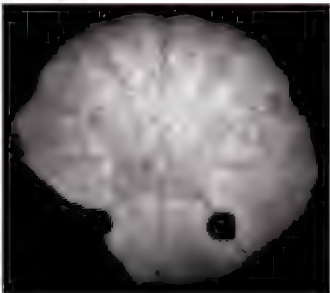



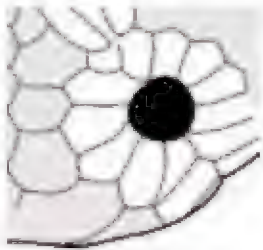
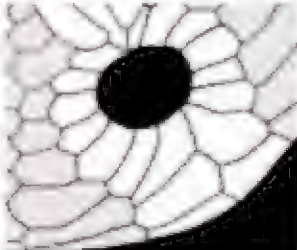
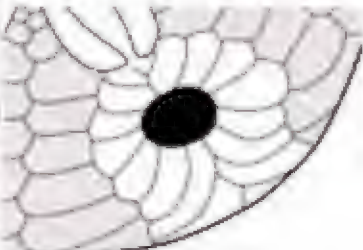



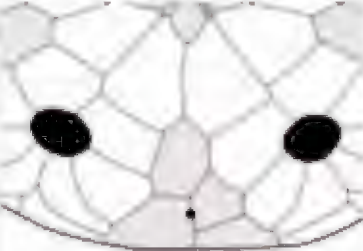
	<i>A. montezemoloi</i>	<i>A. ludovici</i>	<i>Amphiope</i> sp. 2	<i>A. romani</i> n. sp.
TL (mm)	109	29	96 (up to 137)	62
TH	9.8	9	12.3	15
TW	105.7	108	104.7	105
Internal structure (X-ray photographs)				
Shape and size of the central hollow	Subpentagonal-starring 47	Subpentagonal 49	sub-pentagonal 50	Subpentagonal-starring 49
Internal test structure	very light in all the structure	Very dense in whole body	well developed	Very light very dense near the margin
Apical disc position (L4)	59.3	59	59.7	57.5
Petalodium size (PL)	50	50	52	52
N (column a) N (column b)	2 3-4	2 2	2 3-4	2-3 2
N tot - interambulacrum 5	13+	10-11	14-15	13-14
Length of plate 5.b.2 (distance from the posterior edge of the occlusion point)	13.5 (28)	16 (23)	mean 15.6 (32.2)	16 (25)
Lunules: WI SI	295 1,2	100 1.1	250 1,3	123 1.2
Lunules outline and distance from the posterior petals tips				
Periproct position (L11)	12	4.6	13	5.6
Oral scheme with the position of the periproct				
Peculiar characters	Test and lunules very large	Test very small and low Very dense internal structure Very few plates in lamb 5 Periproct very close to the posterior test margin	Test very large	Internal structure very light

Table 2/2. Comparison of the main morphological features of the species of *Amphiope* attributed to the informal bioculata group. If not otherwise indicated, the data report the mean value of the biometric measures expressed as % TL. N = number of post-basical plates in the oral interambulacrum 5, N tot = whole number of plates in the interambulacrum 5. Red numbers = maximum values; blue numbers= minimum values.

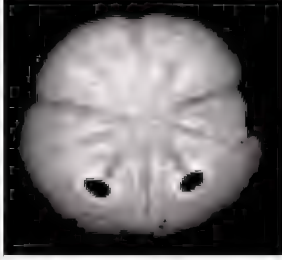
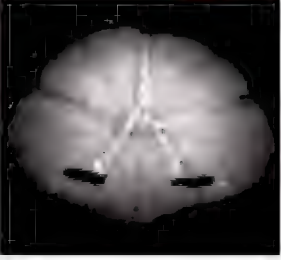
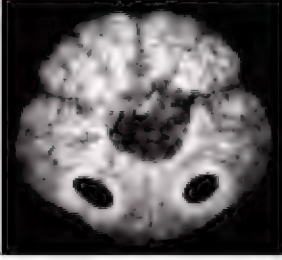
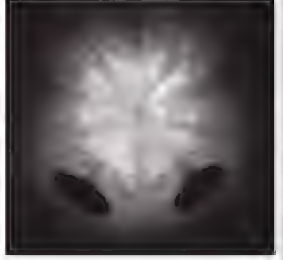



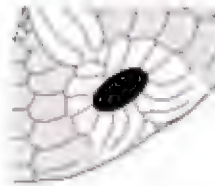







	<i>A. sarasini</i>	<i>A. deyrieri</i>	<i>A. transversifera</i>	<i>A. nuragica</i>	<i>A. pallavicinoidi</i>
TL (mm)	72	51	46	93	100
TH	14	12.5	13	14	11
TW	108.5	122	110	106	111
Internal structure (X-ray photographs)					
Shape and size of the central hollow	Subpentagonal 46	roundish to star shaped	Roundish	Roundish	Subpentagonal
Internal test structure	Light - denser near the margin	Light	Light also near the margin	Strong and dense	Light - denser near the margin
Apical disc position (L4)	57	56	58	57	62
Petalodium size (PL)	49	53	55	53	59
N (column a) N (column b)	2 3	2 2	2 3	3 4	2 3
N tot -interambulacrum 5	13-14	10-11	13	16-20	15-16
Length of plate 5.b.2 (length of the post-basiconal oral interambulacrum 5)	14.6 (28)	18 (25)	18 (27.5)	15 (30)	16.6 (25.4)
Lunules: WI SI	97 1.8	113.5 2.3	149 2	175 2.3	151 2.4
Lunules outline and distance from the posterior petals tips					
Peristome position (L12)	51	56	-	52	66
Periproct position (L11)	12	6-13	7.4	9	9
Oral scheme with the position of the periproct					
Peculiar characters	Very small lunules Strongly branched food grooves	Test very small Low whole number of plates in lamb 5	Test very small Lunules large (highest value of WI in this group)	Shell thick internal structure very strong Poriferous zones convex High number of plates in the oral interambulacrum 5	Test low Petalodium very large

Table 3/1. Comparison of the main morphological features of the species of *Amphiope* attributed to the informal *A. bioculata* group. If not otherwise indicated, the data report the mean value of the biometric measures expressed as % TL. N = number of post-basiconal plates in the oral interambulacrum 5, N tot = whole number of plates in the interambulacrum 5. Red numbers = maximum values; blue numbers= minimum values.

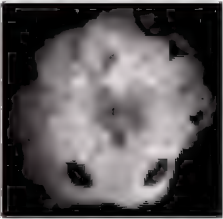









	<i>A. depressa</i>	<i>A. palpebrata</i>	<i>A. tipasensis</i>	<i>A. hollandei</i>	<i>Amphiope</i> sp. 1
TL (mm)	95.5	90	92	106	95
TH	16	14	13	6.5	20.7
TW	111	109	118	120	109
Internal structure (X-ray photographs)	-		-	-	
Shape and size of the central hollow	-	Subpentagonal to roundish	-	-	Subcircular to subpentagonal 47
Internal test structure	-	very light, it becomes denser towards the margin	-	-	Light; become denser near the margin
Apical disc position (L4)	51.5	55.5	-	56	53
Petalodium size (PL)	44	48-51	46	54	54.5
N (column a) N (column b)	-	>2 >2	3 4	3 3	2 3
N tot -interambulacrum 5	-	>14	15-17	14-15	14-16
Length of plate 5.b.2 (distance from the posterior edge of the occlusion point)	-	13.3 (25?)	13 (27,5)	13.2 (26)	15,8 (25)
Lunules: WI SI	57 1.9	91 2.1	99 2.1	72 7.3	184 2
Lunules outline and distance from the posterior petals tips	-				
Peristome position (L12)	-	-	50	60	48
Periproct position (L11)	13	8.7	12.5	12	13.8
Oral scheme with the position of the periproct	-				
Peculiar characters	Test very large Apical disc subcentral with 5 gonopores Petalodium small	Edge of the lunules salient	Test outline roundish High number of plates in the oral interambulacrum 5	Test very low Lunules very long and narrow Frontal petal very long	Test very high Frontal ambulacrum much longer than the others Lunules close to the tips of the posterior petals

Table 3/2. Comparison of the main morphological features of the species of *Amphiope* attributed to the informal *A. bioculata* group. If not otherwise indicated, the data report the mean value of the biometric measures expressed as % TL. N = number of post-basiceoral plates in the oral interambulacrum 5, N tot = whole number of plates in the interambulacrum 5. Red numbers = maximum values; blue numbers= minimum values.

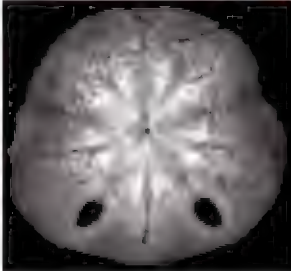
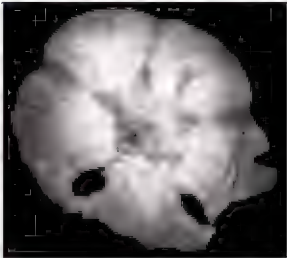





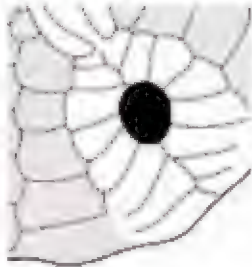





	<i>P. agassizi</i>	<i>P. arcuata</i>	<i>P. cherichirensis</i>	<i>P. baquiei</i>	<i>P. raimondii</i>
TL (mm)	46	54	75	29	53
TH	12.5	9	10	10	11
TW	110	102	123	110	109
Internal structure (X-ray photographs)					
Shape and size of the central hollow	Subpentagonal 48	-	Subpentagonal >46	Subpentagonal 46.5	Subpentagonal 47
Internal test structure	Rather light, denser posteriorly	-	Very dense	Rather light Very dense towards margin	Rather light dense towards margin
Petalodium size (PL)	48	44	50	46.5	42
N plates (column a) N plates (column b)	2-3 2-3	2 2-3	2-3 3	2 2	2 2
N tot - interambulacrum 5	14-15	-	-	10	-
Length of plate 5.b.2 (length of the post- basicoronal oral interambulacrum 5)	16 (26)	14 (24,7)	>18	17,5 (24,3)	15 (25)
Lunules: WI SI	106 1.6	80 1.6	140 1.4	140 1.2	133 2.8
Lunules outline and distance from the posterior petals tips					
Peristome position (L12)	49.5	49	60	51	48
Periproct position (L11)	4.7	9.7	≈10	8.2	7
Oral scheme with the position of the periproct					
Peculiar characters	Basicoronal circle very large (L13= 19% TL)	Test low	Test large Internal structure very dense	Test very small, lunule sub- rounded	Test outline rounded Lunules slit-like and very elongate (SI=3.2)

Table 4. Schematic comparison of the species attributed to the genus *Paraamphiope*. If not otherwise indicated, the data report the mean value of the biometric measures expressed as % TL. N = number of post-basicoronal plates in the oral interambulacrum 5, N tot = whole number of plates in the interambulacrum 5. Red numbers = maximum values; blue numbers= minimum values.

PALAEOECOLOGY

Amphiope, *Paraamphiope* and *Sculpsitechinus* were deposit feeders (Stara et al., 2012; Stara & Sancier, 2014), well adapted to shallow water settings, with middle to high water energy and tropical climate (Stara & Borghi, 2014). They lived partially burrowed, with the petalodium emerging from the bottom. *Amphiope* is commonly found within sediments made of coarse sand and small rounded pebbles, typical of very shallow environments (Stara et al., 2012). Also *P. agassizi* has been cited by Chauzac & Roman (1994) from the late Oligocene of Aquitaine (France), within very shallow near-shore settings, based on the abundance of the tropical bivalve *Trisidos* Röding, 1798 (Arcidae), which is known to live at 2–10 m depth (Chauzac & Roman, 1994). The sediments embedding the fossils under study indicate that *Paraamphiope* and *Sculpsitechinus* were able to settle also finer substrates thus indicating somewhat deeper settings.

This matches with the ecology of the Recent *S. tenuissimus* and *S. auritus* which are known to live at 2–10 m (Lembeh, North Sulawesi, Indonesia, and Tulear, Madagascar, respectively) and, on the other hand, with *Sculpsitechinus* sp.1, which lives at 80 m (Stara & Sancier, 2014). *Sculpsitechinus* cf. *auritus* is common at about 2 m depth at Ghubbet Soghra (Red Sea) but was found also down to 100 m depth (Dollfuss & Roman, 1981).

PHYLOGENESIS AND MIGRATION PATHWAYS

Phylogenesis

Notwithstanding the high number of species (and specimens) examined, the fossil record from Europe, North-Africa and Middle-East of *Amphiope*, *Paraamphiope*, *Echinodiscus* and *Sculpsitechinus* remains patchy, thus preventing to propose reliable hypothesis on the phylogenesis and migration pathways of these genera. Another problem affecting the phylogenetic reconstruction is the uncertainty about the stratigraphical position of a large part of the finding localities, as seen in previous chapter.

Amphiope was able to modify its morphology to adapt to environmental changes and to different set-

tings encountered along the migration pathways. These modifications, which were likely rather quick at the geologic scale, sometimes consisted of back-modifications, thus making difficult the comprehension of the evolutive trends. Also the main trends indicated in Stara & Borghi (2014), when studying *Amphiope* from Sardinia, must be used with caution, since they could be partially obliterated by the still unclear evolution of this genus. They were:

- increasing complexity and progressive reduction of the internal support system; in particular, the dense and strongly developed internal buttress and the thick shell of the late Oligocene-Aquitainian *A. nuragica* was regarded as a primitive character in *Amphiope* with transverse lunules;
- decreasing whole number of plates in the ambulacral (extra petals) and interambulacral columns;
- progressive migration of the periproct towards the peristome, from plates 5.b.3–5.a.3 in *A. nuragica*, to the distal part of the suture separating plates 5.a.2–5.b.3 in *A. lovisatoi* (Burdigalian), to the proximal part of 5.a.2–5.b.3 in *A. montezemoloi* and *Amphiope* sp. 2 (early Langhian).

The factors leading to the noticeable dimensional changes in the size of the petalodium (PL ranges from about 41 to 62% TL in *Amphiope*) are unclear (Stara et al., 2015). However, the differences observed between *A. nuragica* (PL=47–56 %TL) and the closely related species from the Tortonian of north Africa (Morocco, our observations) and Calabria (PL=40.5–46% TL) (Stara et al., 2015) have been interpreted as adaptations to different environmental and climatic changes. Interestingly, the main change in the petalodium size occurred mainly in the Late Miocene (Tortonian) of the southern Proto-Mediterranean area.

The first records of *Amphiope* are dated to the late Oligocene (Chattian)-early Aquitanian of Sardinia and the Early Miocene (Aquitainian) of the Gulf of Biscay, whereas *Echinodiscus pedemontanus* and *Paraamphiope agassizi* were already present in the Oligocene (Rupelian) of the Piedmont Basin and the Gulf of Biscay, respectively; thus they could be possible ancestors of *Amphiope*.

However, some cases point to a different and more complex history. *Paraamphiope agassizi* (Rupelian-Chattian), *A. ovalifera* (early Aquitanian) and *A. romani* n. sp. (Serravallian-Tortonian) are three species from western France, listed in the chronological order. *Amphiope ovalifera* has a

higher whole number of plates in the ambulacral and interambulacral columns than *P. agassizi*. The high number of plates was considered as a primitive feature in *Amphiope* by Stara & Borghi (2014), thus making unlikely a derivation of *A. ovalifora* from *P. agassizi*. *Amphiope ovalifora* shares many features with *A. bioculata* (Langhian-Serravallian of Hérault), notwithstanding the relevant geographic and chronological distance.

Amphiope ovalifora and *A. bioculata* differ by several aspects from *A. sarasini* and *A. lorioli*, which are stratigraphically more recent.

The most intriguing case in *Amphiope* is represented by *A. sarasini*, which shows some intermediate features between *Amphiope* and *Sculpsitechinus*. In the best preserved specimen, the food grooves are rather thin as in *A. bioculata* (Fig. 75), *A. ovalifora* (Fig. 72) and *A. lovisatoi* (Fig. 70) but they are much more branched.

In *Amphiope*, the highest number of post-basicoronal plates in the oral interambulacrum 5 is present (Stara & Borghi, 2014) in the “ancient” *A. nuragica*, with three and four plates in columns “a” and “b” respectively, rarely four and four (Fig. 36). It was also commonly observed that the number of plates decreased in the more recent species (Figs. 40, 42). However, *A. sarasini* has two or three plates in column “a” and three in column “b” (Fig. 44); since it was from the Serravallian-Tortonian, a lower number of plates would be expected. This is valid also for *A. tipasensis*, from the Pliocene of Algeria which has three and four plates in columns “a” and “b”, respectively (Fig. 154). However, the morphological variability is not known yet in this species, as well as its apical disc.

In *A. sarasini*, *Amphiope* sp.1 from Valencia (Stara et al., 2015) (also in this case the apical disc is unknown), *A. lorioli* and *A. tipasensis* the mean distance of the periproct from the posterior test margin (L11) is about 12–13% TL, which represents an elevate value for *Amphiope* (*Amphiope* sp.1 from Valencia has L11=20) and is close to the lowest limit (L11>13) for *Sculpsitechinus*, which gets up to 26% TL (Stara & Sanciù, 2014).

On the other hand, in some Recent specimens of *Sculpsitechinus* the plate 5.b.2 in the oral interambulacrum is in contact with the two adjacent ambulacral post-basicoronals. The occasional occurrence of this character, which is unusual for *Sculpsitechinus*, likely points to a possible relation-

ship between this genus and the latest species of *Amphiope*, such as *A. sarasini*, *Amphiope* sp.1 from Valencia and *A. tipasensis*. All these observations likely indicate the presence of at least three evolutive lines in the genus *Amphiope*:

- *Amphiope elliptica* (Aquitanian-Burdigalian, south-eastern France) and similar species with roundish lunules from the western Proto-Mediterranean Basins (including *A. ovalifora*, *A. bioculata* and *A. romani* n. sp.).

- *Amphiope nuragica* (Chattian-Aquitanian of Sardinia) and its related forms with transversely elongate lunules.

- a group of species, including *A. sarasini*, *Amphiope* sp.1 from Valencia, *A. lorioli* and *A. tipasensis*, with small rounded or narrow lunules, three to four or more numerous post-basicoronal plates in the oral interambulacrum 5 and the periproct far from the posterior test margin (L11>12–13% TL); these forms likely shared a common ancestor with *Sculpsitechinus boulei*.

Paraamphiope agassizi (Oligocene) could be the ancestor of a series of species characterized by small-sized test, the structure in the interambulacrum 5 typical of this species, food grooves large and strongly branched: *P. cherichirensis*, *P. arcuata* (may be also its closely related species from Egypt and Libya), *P. baquiei* and the Recent *P. raimondii*.

Sculpsitechinus boulei (Aquitanian) is the earliest species so far known of this genus. However, likely it was not the common ancestor of all the recent species of *Sculpsitechinus* (Figs. 80, 81), since some specimens of *S. auritus* have a higher number of plates in the whole interambulacrum 5 and in its oral part (4/4) (Stara & Sanciù, 2014; pl. 11, figs. 1–4, 6–7). Therefore, the presence of an earlier taxon (from Oligocene?) is prospected, which was the common ancestor of *S. boulei*, *S. auritus* (with 4/4 plates and slit-like notches) and the group of species of *Amphiope*, including *A. sarasini* and *A. tipasensis*, with small transverse lunules and 4/4 plates in the oral interambulacrum 5.

However, only a cladistic analysis based also on the results of the other researches in progress in other peri-Mediterranean areas will help to clear the complex phylogenesis of this genus.

Migration pathways

The appearance of the first lunulate scutelliforms in the Atlantic-Europe and the Western Proto-

Mediterranean (sensu Stara & Rizzo, 2014) followed the great extinction occurred at the Eocene-Oligocene transition (see Miller et al., 2009, with references). The renewal of the scutelliform fauna after that critical period was likely the source of the ancestors of *Amphiope*, *Paraamphiope* and, maybe, also of *Echinodiscus* and *Sculp-sitechinus*.

Although the first citations of *Amphiope* date back to the Late Oligocene-Early Miocene in the area comprehending Sardinia, Gironde and Provence, the genus at that time was already well differentiated, thus pointing to an earlier origin well into the Oligocene. In fact, *A. nuragica* (late Chattian-early Aquitanian of Sardinia), *A. ovalifera* (early Aquitanian of Gironde) and *A. elliptica* (late Aquitanian of the Rhône Basin) were morphologically very different.

Amphiope diffused from that area towards the southern and eastern parts of the Proto Mediterranean (Fig. 1, pro parte) and western Africa (de Loriol, 1905; Darteville, 1953).

The investigation of the migration pathways is affected by the same problems seen in the phylogenetic analysis, that is patchy fossil record and uncertainty regarding the stratigraphic position of a part of the fossil material and finding localities. However, a useful tool for the investigation is provided by the palaeoecology of this echinoid: *Amphiope* was a strictly shallow water echinoid, thus its diffusion occurred only through nearshore settings and it was not able to cross large deep-water basins.

The case of Sardinia corroborates this hypothesis, since the species described from that area from late Chattian to the early Messinian (Stara & Borghi, 2014) were never recorded from other areas, thus indicating the isolation of that isle with regard to the local populations of *Amphiope*. Sardinia shifted away from the European plate starting from the end of the Oligocene - beginning of the Miocene (Stara & Rizzo, 2014, with references), and was transformed by the progressive marine incursion into an archipelago emerging from an epicontinental sea (Gattaceca et al., 2007). In that period *Amphiope* was represented in Sardinia by *A. nuragica* and *Amphiope* sp. 1, which were morphologically very different from *A. elliptica* living in the Provençal Basin during the Aquitanian, thus pointing to an earlier separation of the populations of the two basins. In the Middle Miocene Sardinia was surrounded by a deep sea which increased the

geographic isolation, favoring the development of new species, such as *A. pallavicinoi*, *A. lovisatoi*, *A. montezemoloi* and *Amphiope* sp. 2 from the Burdigalian-early Langhian of the Calcarei di Mores Formation, which were different from the coeval species in south-eastern France. In particular, small to medium-sized forms prevailed in the French basins, whereas the average size was much larger in Sardinia (TL up to 170 mm). These differences were likely due to more favorable environmental conditions for *Amphiope* present at that time in Sardinia.

Data regarding the Balearic Islands, the Spanish coasts and the Kabylies were not available to this study and the plate patterns in *Amphiope* from Sicily (Garilli et al., 2010) must be improved; however, a new research is in progress on these arguments.

The diffusion of *Amphiope* starting from the original area was rather quick, since in the ?Burdigalian it was already present in Angola (de Loriol, 1905) and in the Paratethys (Hoernes, 1883). Also in the eastern Proto-Mediterranean, specimens attributable to the *Amphiope nuragica* group were collected from the Burdigalian of Erzincan (MNHN-F, R67283, collection Pinar), along the Turkish southern coast of the Black Sea, and from the Mut Basin (southern Turkey; Kroh & Nebelsick, 2003).

Some specimens of *Amphiope* from Mosul (Iraq, NHMUK.E73263) and the Gulf of Aqaba (Jordan, NHMUK.EE534-8) and those from the Qom Formation in Central Iran (Khaksar & Moghadam, 2007), indicate the presence of populations attributable to the *Amphiope nuragica* group in the Early Miocene of eastern Asia. Those forms certainly migrated when the connection between the Paratethys or the eastern Proto Mediterranean and the Indian Ocean was still active, that is before the definitive closure of that channel occurred in the Middle Miocene between 17 and 11 Ma (Hüsing et al., 2009). In particular, the specimens of *Amphiope* from Iran were dated to the late Aquitanian (Khaksar & Moghadam, 2007), thus indicating a quick arrival from the original area.

The diffusion of *Amphiope* from the western towards the eastern part of the Proto Mediterranean was controlled by the Alpine orogeny which periodically opened peri-Alpine channels connecting the two parts of the Basin, during the Early Mio-

cene. Some studies (e.g. Rögl et al., 1998; Harzhauser et al., 2007) affirmed that during the Oligo-Miocene the connection between the western and eastern parts was wide and continuous. However, a different theory suggests that the two parts of the Basin were only occasionally connected (Rosembaum et al., 2002; Stara & Rizzo, 2013; 2014).

The citation of a single species (*A. tipasensis*) from the Pliocene of western Mediterranean, represented by a sole and incomplete specimen, indicates that *Amphiope* survived the Messinan crisis, likely along the western Atlantic coasts, and re-entered the Mediterranean though with a strong reduction of diversity. The citation of a number of *Amphiope*-bearing outcrops from the Atlantic coast to the Taza-Guercif basin of the Morocco Late Miocene gateway (Lacointre, 1952; Flecker et al., 2015) and a study in progress on some specimens (MNHN-F R67286) from the Middle-Late Miocene of the Guercif area, corroborate this hypothesis.

A more accurate reconstruction will be provided by the results of the investigations in progress on unpublished fossil findings from southern and central Italy, Spain (including the Balearic Basin), Portugal, Morocco, Algeria and Libya.

CONCLUSIONS

Recent studies (e.g. Stara & Borghi, 2014) pointed out that a revision by modern methods of the earlier species attributed to *Amphiope* was needed to clear the largely unresolved taxonomy of this genus. In particular, the distinctive characters of the type species, *A. bioculata* (Des Moulins, 1837), were so far unclear and the type locality was unknown. The Serravallian of the surroundings of Nissan (Hérault, France) has been here considered as the type locality for *A. bioculata*, following the opinion of earlier studies (de Loriol, 1902; Cottreau, 1914; Lambert, 1912a; 1915a). Since the existing type was badly preserved, the re-description of the type-species has been based on a sample made of 11 well preserved topo-typic specimens from Lespignan and Nissan.

The type material of several species of *Amphiope*, examined in public institutions, and new topo-typic specimens, studied using statistical biometrical analyses and with emphasis on the test structure, enabled comparison between a relevant

part of the earlier species attributed to this genus.

On the whole the research indicated that apparently similar forms in this genus could turn out to have a different structure and, consequently, to represent well distinct species; therefore, the high morphological variability, which is objectively typical of *Amphiope*, cannot justify the institution of new species, based only on external characters.

As a result (Table 1), 17 species have been confirmed as valid and maintained in the genus *Amphiope*; *A. romani* n. sp. has been instituted on the basis of a group of specimens from the late Serravallian-early Tortonian of Channay-sur-Lathan, Touraine (France). Three species previously assigned to *Amphiope* have been transferred to the genus *Paraamphiope* (Stara & Sanciù, 2014), since they have radially elongate lunules separated by at least three to four plates from the posterior petals tips, small or rather small petalodium and strongly branched oral food grooves. Another species with very branched food grooves, radially elongate lunules separated from the posterior petals by four plates and with a oral interambulacral plating consistent with that of *Sculpsitechinus tenuissimus*, has been transferred to the genus *Sculpsitechinus* (Stara & Sanciù, 2014). *Amphiope arcuata* Fuchs, 1882 have been recently transferred to the genus *Paraamphiope* by Stara & Sanciù (2014); the generic placement of three similar species from the Miocene of Libya and Egypt, *A. truncata* Fuchs, 1882, *A. fuchsi* Fourtau, 1899 and *A. miocenica* Ali, 1998, remains uncertain since no plating schemes were provided in the original descriptions. The attribution of these species to *Paraamphiope* seems more probable, due to their close similarity with *P. arcuata*. For an overview, the results of this work have been condensed in Tables 2–4.

The original description of a number of other species attributed to *Amphiope* did not provide reliable distinctive characters and in some cases information on the repository of the type material and on the type localities was not reported. This was the case with *A. perspicillata* Agassiz, 1841, *A. styriaca* Hoernes, 1883, *A. personnata* Pomel, 1887, *A. villei* Pomel, 1887, *A. dessii* Lovisato in Cotteau, 1895, *A. laubei* Lambert, 1912, *A. calvii* Lovisato, 1914, *A. labriei* Lambert, 1915 and *A. dallonii* Lambert, 1931, which are here considered as *species inquirendae*.

New morphological acquisitions regarding the characters of *Amphiope* and *Paraamphiope* are:

- almost the same whole number of plates is present in each column of the interambulacrum 5 and the ambulacra I and IV (sometime one or two plates of difference).

- the species represented by large samples from a single locality often show a rather wide morphological variability regarding above all shape and size of test, petals and lunules, whereas the patterns and the features of the internal support system are almost constant.

The last point indicates the need of basing the study of these astriclypeids on samples made of a significant number of specimens. Only in a few cases we accepted the validity of species represented by a sole specimen (e.g. *A. hollandei* and *A. transversifora*), since the types showed peculiar features and looked well characterized. It would be desirable, however, that new topotypic material could clear the morphological variability of these two species, which remains so far unknown.

Notwithstanding the news provided by the present research, the fossil record of *Amphiope* and the other closely related genera, *Paraamphiope*, *Echinodiscus* and *Sculpsitechinus*, remains patchy, thus preventing to propose exhaustive hypothesis about the phylogenesis and the migration pathways of these astriclypeids. For this purpose, new studies are needed to clear the partially unresolved stratigraphy of a large part of the finding localities examined in this work. Studies in progress dealing with the populations of *Amphiope* from Italy (Calabria, Campania and Tuscany), Spain, Portugal and north Africa (Algeria, Morocco) and of *Sculpsitechinus* from Iran will likely provide new useful data.

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Proceedings of the 17th International
Colloquium on Amphipoda (17th ICA),
September 4th-7th 2017, Trapani (Italy)



Parhyale plumicornis (Heller, 1866) - Amphipoda Hyalidae

The 17th International Colloquium on Amphipoda

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ABSTRACT

The 17th International Colloquium on Amphipoda (17th ICA) has been organized by the University of Palermo (Sicily, Italy), and took place in Trapani, 4-7 September 2017. All the contributions have been published in the present monograph and include a wide range of topics.

KEY WORDS

International Colloquium on Amphipoda; ICA; Amphipoda.

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Proceedings of the 17th International Colloquium on Amphipoda (17th ICA), September 4th-7th 2017, Trapani (Italy)

The first International Colloquium on Amphipoda was held in Verona in 1969, as a simple meeting of specialists interested in the Systematics of *Gammarus* and *Niphargus*.

Now, after 48 years, the Colloquium reached the 17th edition, held at the “Polo Territoriale della Provincia di Trapani”, a site of the University of Palermo, in Italy; and for the second time in Sicily (Lo Brutto et al., 2013).

The Organizing and Scientific Committees were composed by people from different countries.

Exclusively from Italy within the Organizing Committee:

Sabrina Lo Brutto (Coordinator) - University of Palermo, Italy

Marco Arculeo - University of Palermo, Italy

Vincenzo Arizza - University of Palermo, Italy

Mirella Vazzana - University of Palermo, Italy

Elvira De Matthaeis - University La Sapienza, Italy

Felicita Scapini - University of Firenze, Italy

Alberto Ugolini - University of Firenze, Italy

Eugenia Schimmenti - University of Palermo, Italy

Davide Iacofano - University of Palermo, Italy.

And from Italy, Germany, Spain, Portugal,

Poland, Turkey, Norway, Brazil and Canada within the Scientific Committee:

Sabrina Lo Brutto (Coordinator) - University of Palermo, Italy

Elvira De Matthaeis - University La Sapienza, Italy

Felicita Scapini - University of Firenze, Italy

Alberto Ugolini - University of Firenze, Italy

Maria Beatrice Scipione - Stazione Zoologica Anton Dohrn, Italy

Murat Özbek - Ege University Faculty of Fisheries, Turkey

Michał Grabowski - University of Lodz, Poland

Oliver Coleman - Museum für Naturkunde, Germany

José Manuel Guerra García - Universidad de Sevilla, Spain

Jan Beermann - Alfred Wegener Institute, Germany

Filipe Costa - University of Minho, Portugal

Ed Hendrycks - Canadian Museum of Nature, Canada

Cristiana Serejo - Universidade Federal do Rio de Janeiro, Brasil

Anne Helene Tandberg - University Museum of Bergen, Norway

Anne-Nina Lörz - CeNak-Universität Hamburg, Germany.

The Honorarium Committee was the one confirmed from the previous meetings:

Claude de Broyer - Royal Belgian Institute of Natural Sciences, Belgium

Faouzia Charfi-Cheikhrouha - University of Tunis El Manar, Tunisia

Krzysztof Jażdżewski - University of Lodz, Poland

Gordan Karaman - Montenegrin Academy of Sciences and Arts, Montenegro

Traudl Krapp-Schickel - Alexander Koenig Research Museum, Germany

Jim Lowry - Australian Museum Sydney, Australia

Ilona Muskó - BLRI, Hungarian Academy of Sciences, Tihany, Hungary

Alan Myers - National University of Ireland, Ireland

Boris Sket - University of Ljubljana, Slovenia

Wim Vader - Tromsø Museum, Norway.

The Colloquium included a great variety of studies on Amphipoda (Systematics, Ecology, Biogeography, Physiology, Genetics, etc.), all presented in 3 plenary lectures, 62 oral communications, and 57 posters, and published in the present issue, thanks to Members of the Scientific Committee, which reviewed the contributions and significantly helped in assessing and improving these proceedings.

There were 120 participants, from the 33 countries (Fig. 1) of which 41% students (MS, PhD or

Post-Doc). The country which the highest number of participants came from was Italy with 24 participants (of these 11 students), followed by Poland (12 participants, of these 4 students), and Tunisia and Germany (9 participants, of these 4 students, both) (Fig. 2). Several students came also from different countries, maybe due to the low cost of the registration fee.

As a matter of fact, the Colloquium gave a particular attention to needs of young students and offered travel grants, thanks to Filipe Costa, under the supervising of Jan Beermann.

The meeting was an opportunity for youngs, as it registered the most authoritative Amphipodologists of the world. On the basis of a quick search on the Web of Science and Scopus databases, we can assert that all the participants have been authors of more than 2,500 papers published in peer reviewed journals, with more than 38,500 citations. The numbers increase if journals without impact factors are included.

The International Colloquia on Amphipoda are commonly a meeting point between various amphipod specialists with a very high scientific impact on academic community and an occasion of transfer information and knowledge.

The 17th International Colloquium on Amphipoda was opened by James K. Lowry & Alan A. Myers; they reported a new classification of Amphipoda, establishing the new order Ingolfiellida (Lowry & Myers, 2017). From the first contributions, it pointed out clearly that our knowledge on



Figure 1. Map of countries which the 17th ICA 122 participants came from.

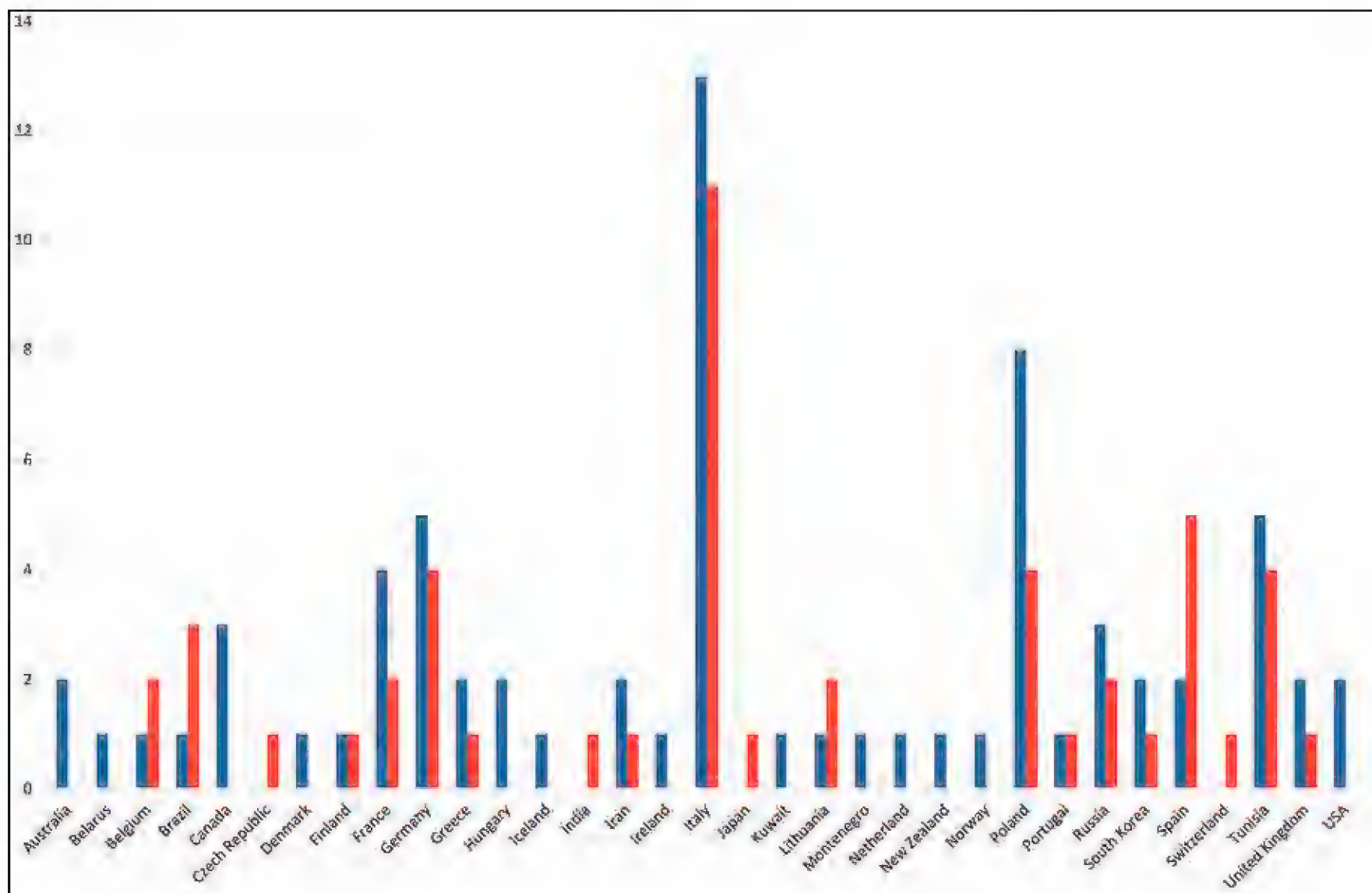


Figure 2. Number of participants per country (blue, regular participants; red, students)

amphipods diversity is still poor (Hughes, 2017; Satyam et al., 2017; Zakhama-Sraieb et al., 2017); and regarding the alien species, the knowledge of this taxon results far from being exhaustive (Cardeccia & Marchini, 2017; Thomas & White, 2017).

During the Colloquium, special attention was given to freshwater amphipods (Alther & Altermatt, 2017; Copilaş-Ciocianu et al., 2017; Hupalo et al., 2017; Šniaukškaitė, 2017), but also to abyssal amphipods (Frutos, 2017; Corbari & Jazdzewska, 2017; Horton et al., 2017) and amphipods inhabiting cold environments (Havermans et al., 2017; Lörz & Jazdzewska, 2017; Verheye et al., 2017). The Colloquium gave its contribution on how we can use the amphipods in aquaculture (Guerra-García et al., 2017). While, the DNA barcode approach and genetic results were also discussed (Vieira et al., 2017; Radulovici & Coleman, 2017).

Authors, and local organizers, focused their efforts in the success of the 17th ICA, and deeply thank all the contributors for being so numerous and enthusiastic.

ACKNOWLEDGMENTS

The University of Palermo organized the 17th ICA, with the partnership of the Italian Zoological Union (UZI, www.uzionlus.it), Italian Society of Marine Biology (SIBM, www.sibm.it) and the Museum of Zoology “P. Doderlein” - SIMUA (Palermo, Italy, <http://museozoologia.unipa.it>).

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Trends in aquaculture today. Marine amphipods as alternative resource

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ABSTRACT

In the framework of the innovative research programmes in aquaculture there are currently two areas of increasing interest: (i) the search for alternative live feed organisms, and (ii) the progress in ‘Integrated Multi-Trophic Aquaculture’ (IMTA). Recent studies have shown that marine amphipods are an interesting potential aquaculture resource for many reasons: (i) they have a widespread global distribution, (ii) they form an important natural dietary component in a variety of coastal marine finfish and molluscs, (iii) they contain high levels of beneficial polyunsaturated fatty acids, high protein content and adequate aminoacid profile, (iv) they are relatively sedentary, readily colonize artificial structures (fouling communities) and under appropriate conditions can reach high biomass, (v) they exhibit fast growth with several generations per year, (vi) they are opportunistic feeders, (vii) some species show wide environmental tolerances, and (viii) they are suitable to larger scale culture, being able to feed on detritus. Therefore, they could be incorporated in IMTA systems to recycle detritus, being potential candidates for bioremediation. At the same time, important densities of amphipods associated to these IMTA systems could be reached; besides the use of these amphipods as live feed, another feasible approach could involve their dehydration for use as a component in the fabrication of fish feed or to be encased in microdiets.

KEY WORDS

Aquaculture; amphipods; IMTA; fish feed; bioremediation; resource.

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Aquaculture production will need to increase considerably to meet future demands. The challenges facing aquaculture are recognized by the European Commission and are addressed through the EU Blue Growth Strategy and the reformed Common Fisheries Policy (Alexander et al., 2015). In this context, the search for alternative live feed organisms, and the progress in ‘Integrated Multi-Trophic Aquaculture’ (IMTA) are main topics. Many marine finfish aquaculture efforts, particularly for

larval or juvenile finfish stages, utilize a limited range of live feed organisms such as: *Artemia*, rotifers, copepods and mysid shrimp (Woods, 2009 and references therein) and there is an urgent need to explore and investigate new aquatic organisms as live feed in aquaculture. On the other hand, great efforts are being made to develop innovative technology IMTA. IMTA involves the integrated cultivation of fed species (e.g. finfish) together with extractive species (marine invertebrates and/or

algae) which feed on detritus from the fed species (Alexander et al., 2015). IMTA allows species from two or more trophic levels to grow simultaneously in the same farm, with the waste of one feeding the other

During the last decade, there is an increasing interest in the potential use of marine amphipods for aquaculture and ornamental aquarium hobby. Marine amphipod have a widespread global distribution and form an important natural dietary component in a variety of coastal marine finfish and molluscs (Baeza-Rojano et al., 2013a; Jimenez-Prada et al., 2015; Calero-Cano et al., in prep). They exhibit fast growth with several generations per year and they are opportunistic feeders, being able to feed on detritus. Woods (2009) conducted a comprehensive review examining aspects of the known biology and ecology of caprellid amphipods and their potential suitability as a novel marine finfish feed. In fact, he pointed out that caprellids could have a beneficial role to play in integrated coastal aquaculture, as a combined bioremediator, feed resource, and macroalgal enhancer. Guerra-García et al. (2006) also found that caprellids are characterized by higher levels of polyunsaturated fatty acids (omega 3 and omega 4) than freshwater amphipods. Traditionally, the commercial use of amphipods has been restricted to freshwater species, used as food for aquarium fishes and turtles (see e.g. <http://www.tropical.pl/en/products/terrarium/foods/gammarus/>). Marine amphipods show a high levels of beneficial polyunsaturated fatty acids as DHA and EPA and adequate protein content (e.g. Moren et al., 2006; Suontama et al., 2007; Hyne et al., 2009; Baeza-Rojano et al., 2014). Baeza-Rojano et al. (2010) demonstrated that gammarideans could be used as an alternative prey to mysids to culture cuttlefish (*Sepia officinalis*) hatchlings. Additionally, Baeza-Rojano et al. (2013a) also reported the successful use of marine amphipod as alternative prey for *Octopus hatchlings* culture. *Octopus maya* hatchlings fed with marine gammarideans showed significantly higher survival rate, growth, and nutrient assimilation compared with hatchlings fed *Artemia* sp. or freshwater gammarideans.

Besides their interesting nutritional composition, amphipods can be cultured at high scales. For example, Nakajima & Takeuchi (2008) reared *Caprella mutica* in an exhibition tank in a public aquarium. González et al. (2011) described the

abundance of exotic amphipods associated with aquaculture systems and reported their interest as animal food, and Baeza-Rojano et al. (2013b) conducted a preliminary study of the *Caprella scaura* amphipod culture for potential used in aquaculture. These cultures can be developed in indoor intensive tank farming, but could be also sustainably developed in extensive cultures of coastal areas or open sea. In this sense, Baeza-Rojano et al. (2013b) suggested the suitability of amphipods to be included in programs of Integrated Multi-Trophic Aquaculture (IMTA), feeding on products of other cultivated species. Guerra-García et al. (2016) demonstrated experimentally that detritus (mainly composed of uneaten feed pellets and fish faeces released by cultured fish in fish farms and sea-cage structure) can be a nutritional adequate and cheap feed for caprellid amphipods, providing a source of both omega-3 and omega-6 fatty acids. Therefore, these authors reinforced the suitability of amphipods (caprellids in this case) to be use in IMTA systems associated with extensive culture of floating farms of fishes or molluscs or with intensive cultures in terrestrial systems (Fig. 1). Recent studies (Jiménez-Prada et al., in prep.) have revealed another interesting example of potential IMTA systems combining the extensive culture of fishes and amphipods associated to the marsh ponds of Southern Spain. Amphipods are naturally cultured in high densities associated to algae and/or other substrates (such as traps or other artificial devices where amphipods can attach see Fig. 1). They feed mainly on detritus (e.g. faeces) produced by the fishes which are growing in the ponds. Thus, a high and sustainable production of amphipod can be obtained. These amphipods could be useful as natural food for fishes cultured in the marsh ponds and as additional resource to be used in aquaculture (alive or liophilized, as a whole or integrated in fish feed). Taking into account that amphipods exhibit fast growth with quick time to reproductive maturity, short interbrood periods, and that they are opportunistic feeders, the use of traps, artificial meshes or cages (see Fig. 1) could increase the available substrate for amphipods to cling on, reproduce and grow. Once these structures have been fully colonized by the amphipods (in a few weeks), they could be withdrew and amphipods removed using freshwater. The ponds are easily accessible from land, and algae, traps or other devices can be placed and replaced without great effort and with low

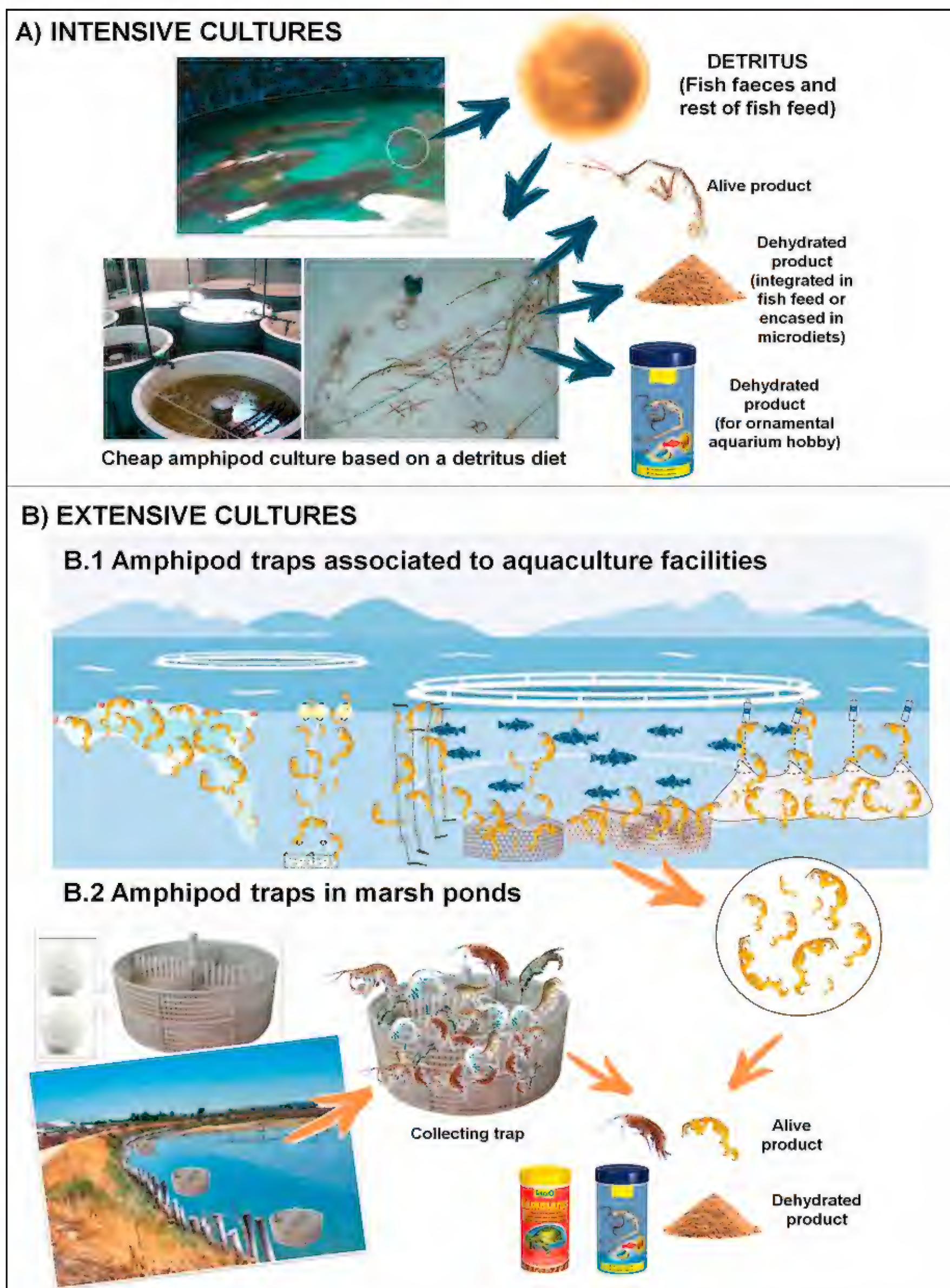


Figure 1. Intensive amphipod cultures in tanks (A) and extensive amphipod cultures in structures (nets, buoys, cages, lines) placed in the aquaculture facilities (B1) or in collecting traps associated to marsh ponds in Southern Spain (B2).

costs. Marsh ponds are, consequently, promising sceneries to develop environmentally sustainable IMTA systems.

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New and little known coastal Talitrids from Australia (Amphipoda Crustacea)

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ABSTRACT

The taxonomy of Australian coastal talitrids is reviewed, with new genera and species recognised, along with the revision of several historic taxa little known since their description in the 1870's. Recently studied collections from Tasmania have provided significant input to distribution records, with these comprehensive surveys including the World Heritage Listed region of south west Tasmania. This new information supports a reconsideration of current evolutionary relationships within and between species and genera of Australian Talitridae.

KEY WORDS

Talitridae; Australia; taxonomy.

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INTRODUCTION

Australia hosts the most diverse Talitrid fauna in the world at both the generic and species level, yet is one of last of the first world countries to document this group. Australia currently has 23% of the known world talitrid species, comparatively Europe host around 9% of the world fauna.

Talitrids were among the earliest amphipod species described in the 1870's by Australia's first Professor in Zoology H.A. Haswell. After this initial work the Talitridae of Australia received little attention until recent times. The coastal talitrids of southern Australia from Western Australia to New South Wales and the terrestrial talitrids of Tasmania and New South Wales have been well-studied (Lowry & Peart, 2010; Serejo & Lowry, 2008; Friend, 1979, 1982, 1987). Five species have been described from Darwin and the Great Barrier Reef (Lowry & Peart, 2010; Serejo, 2009; Lowry & Springthorpe, 2009a,b). The coastal areas of tropical Australia from the Kimberley to Kakadu have

begun to be described (Lowry & Springthorpe, 2015). The coastal beaches and salt marshes of Tasmania are undocumented except for five species and are the focus of current study (Hughes & Ah Yong, 2017; Richardson, 1993, 1996).

RESULTS AND CONCLUSIONS

With the recognition of new species of talitrid from Tasmania, this area now holds the highest species diversity for Talitrids in Australia. Additionally, the study of several historic taxon has recognised junior synonyms (*B. pravidactyla*, Hughes & Ah Yong, 2017) and led to an updated generic classification of some species (Hughes & Lowry, A submitted). New genera have been established which highlight more novel sexually dimorphic characters in male within the family Talitridae (Hughes & Lowry, B accepted). Material contributing to these findings were the extensive collections associated with ecological surveys led by A.M.M. Richardson

(Richardson et al., 1991, 1997). Of most significance, in terms of location and diversity, are collections from the south west region of Tasmania which is a World Heritage List area.

Current studies demonstrate that characters such as the presence and/or number of robust setae on the uropods and telson are variable with growth stage in some Australian talitrid species (Hughes, submitted). These characters have previously been used to define genera. This outcome highlights the need to reconsider how these characters are applied to infer generic level relationships.

Based on both the newly identified species and development of character states, it is evident that the current generic classification of Australian talitrid fauna does not adequately reflect the group. Broader taxonomic changes to encapsulate the newly recognised diversity still require further study to investigate the relationship of Australian genera with surrounding regions.

ACKNOWLEDGEMENTS

The study was made possible by an Australian Biological Resources Study Postdoctoral grant RF 215-51. I am grateful to Jim Lowry for his ongoing support towards the study of Australian Talitrids which made this project possible. I thank Alastair Richardson for making his Tasmanian collections available for study and the collection management staff in the Australian Museum Marine Invertebrates section for curation of material.

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Variation of orientation adaptation in Talitrids

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ABSTRACT

Talitrids are model species for the analysis of orientation behaviour. Comparative studies were carried out on various species on Mediterranean and Atlantic beaches to understand the cues and mechanisms used to orient and the sources of variation of this behavioural adaptation. An overview is presented on an extended data set that allows for general considerations.

KEY WORDS

Orientation behaviour; Mediterranean and Atlantic beaches; sand-hoppers; beach-hoppers.

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INTRODUCTION

Pardi & Papi (1952) first reported the astromonic orientation (sun and moon compass) capability of sand hoppers and proposed it as a general adaptation of littoral talitrids, to recover the optimal zone on their home beach. This fostered research both in the laboratory and field, to understand the physiological mechanisms at the basis of this behaviour, which assessed the sensorial capabilities and orientation responses to various stimuli (reviews in: Scapini, 2006; Scapini et al., 2013). A comparative approach on populations from different beaches was used to integrate the experimental one and analysed the variation related to environmental variability. I focus here on the comparative approach on talitrid orientation to recover the preferred zone on the beach.

MATERIAL AND METHODS

To compare behavioural expression, the orientation of individuals was tested under similar conditions, both internal (regarding the animal itself) and external (environmental). The minimum ex-

perimental disturbance was applied on freshly collected individuals and suitable numbers of replicates were made, to compare the distributions of different populations. Replicates were considered the individuals released under similar conditions, using small groups, as orientation was shown not to be influenced by the presence of conspecifics (Scapini et al., 1981). The individuals were released in the centre of dry Plexiglas arenas of 40 cm diameter, horizontally positioned, with 72 pitfall traps at the rim, permitting the view if the sky and sun, with landscape view permitted or screened off (experimentally fixed factor). The meteorological variables that could influence behaviour were recorded at each release. Experiments were made at different times of the day (morning and afternoon), to analyse the response to azimuthal change (experimental variable). The distributions obtained were analysed through a multiple regression analysis, adapted to circular distribution (Marchetti & Scapini, 2003). The response variable was the orientation expressed by individuals; the best models for each population distribution were obtained, with the minimum number of parameters and the maximum likelihood.

RESULTS AND CONCLUSIONS

The meta-analysis of the orientation under similar test conditions (as described above) of several populations from different talitrid species (both sand-hoppers and beach-hoppers) and different coasts (northern and southern Mediterranean, eastern and western Atlantic Ocean) showed a common orientation seawards as general pattern. Also the variation due to intrinsic and environmental factors and variables revealed similar patterns, with smaller individuals (young) showing a more precise seawards orientation than bigger ones, and predictable effects of air temperature and humidity. Dry conditions and higher temperatures elicited a prompter and more precise seaward orientation, confirming its survival function, to avoid dehydration risk during sunny days. It can be concluded that sun orientation is a general adaptation of beach talitrids. On the other hand, the variation observed among populations depended on the time of day, landscape vision and geo-morphological features of the beach at the site of collection (slope, extension, presence of wrack on the beach human disturbance). The responses to such factors are likely learned by each population or sub-population under natural conditions, so that their use in

orientation becomes opportunistic, i.e. depending on the circumstances.

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I wish to thank the students and researchers who have participated in these experiments

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***Sicafodia iceage* Jimenez Campean et Coleman, 2017 (Crustacea Amphipoda Sicafodiidae) from the North Atlantic**

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ABSTRACT *Sicafodia iceage* from the North Atlantic is compared to the only other species from then genus *Sicafodia stylos*.

KEY WORDS Taxonomy; North Atlantic; Tasman Sea; *Sicafodia iceage*; *Sicafodia stylos*.

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During the ‘IceAGE 2 amphipod identification work-shop’ (IceAGE = acronym for ‘Icelandic marine Animals: Genetics and Ecology’) in Wilhelmshaven, Germany in summer 2016, we encountered an unusual amphipod species collected in deep water of the North Atlantic. The species turned out to belong to the monotypic family Sicafodiidae Just, 2004. This taxon is unusual in two respects:

(1) Sicafodiids have conically bundled, elongate, pointed mouthparts that might have a piercing and sucking function. Pointed and conically bundled mouthparts are rare within amphipods. They also occur in Acanthonotozomatidae Stebbing, 1906, Ochlesidae Stebbing, 1910, Pardaliscidae Boeck, 1871, within lysianassoids in the genus *Acidostoma* Lilljeborg, 1865, and very similarly developed as in the Sicafodiidae in some genera of Iphimediidae Boeck, 1871 (e.g. *Parapanopea* Nicholls, 1938).

(2) The only hitherto known species from this family, *Sicafodia stylos* Just, 2004, was collected in the Pacific Ocean, Tasman Sea, 27 km east of Tasmania. Just (2004) tried to classify the species within the known amphipod families, discussed its affinities to Ochlesidae, Lafystiidae, Laphystiopsidae and Pardaliscidae, but decided to create a new family for it, as he was not able to include this species in any of those families.

Now, we have found material of Sicafodiidae (Fig. 1) from the North Atlantic, 17,000 km away from the first locality. It is somewhat similar to Just’s (2004) species, but differs in mouthpart characters and the width of the coxa of pereopod 5. Because of these differences and the disjunct distribution of the two species, we consider this material as a species new to science (Jimenez Campean & Coleman, 2017) and named it *Sicafodia iceage*.

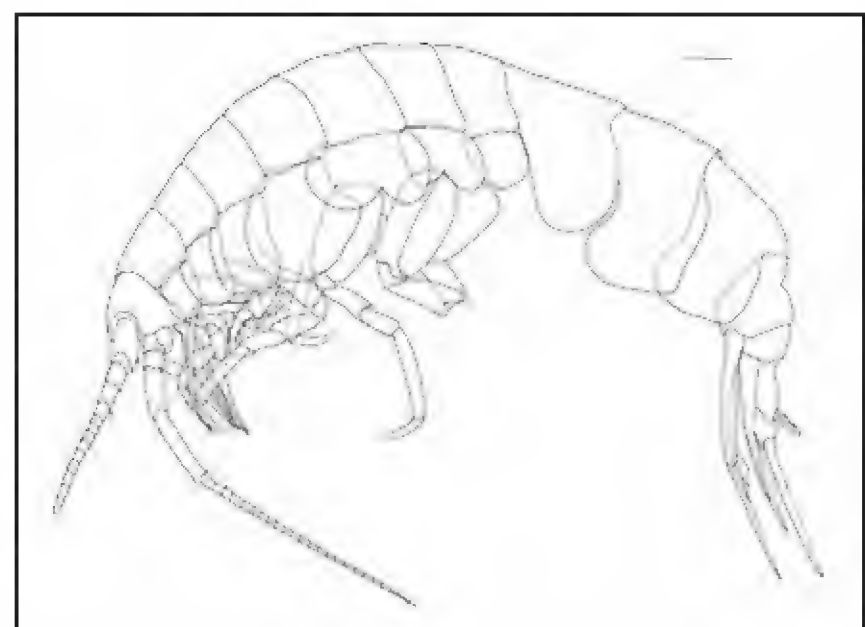


Figure 1. *Sicafodia iceage*, holotype female, 10.5 mm.
Scale bar: 500 µm.

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Independent and recent large-scale dispersal into surface waters by two species of the subterranean amphipod genus *Niphargus* Schiødte, 1849 (Niphargidae)

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ABSTRACT

Due to the fragmented nature of groundwater habitats, groundwater fauna is characterized by poor dispersal and narrow endemism. Although *Niphargus* Schiødte, 1849 (Niphargidae) comprises hundreds of narrowly endemic groundwater species, a handful occur in surface waters, and two of these, *N. hrabei* and *N. valachicus*, have very large ranges (>1300 km). We hypothesize that these two species were able to secondarily colonize surface habitats and that this ecological shift facilitated large-scale dispersal. Despite their ecological and biogeographic similarities, phylogenetic analyses revealed that they are not closely related and independently colonized surface waters from groundwater ancestors. Their phylogeographies reveal dispersal episodes throughout the Danube lowlands during the Pleistocene, and their morphologies are closer to each other than to most other epigean *Niphargus* species. In conclusion, our results indicate that adaptation to groundwater is not a one-way evolutionary path and that groundwater species can occasionally recolonize and widely disperse in surface waters.

KEY WORDS

Dispersal; ecological shift; morphology; phylogeny; phylogeography.

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Groundwater is an extreme and highly fragmented habitat. Consequently, groundwater species are highly specialized and restricted to the subsurface, and are therefore unable to disperse throughout the relatively well-connected yet competitively demanding epigean environment. As such, groundwater fauna is characterized by a high level of endemism and high beta diversity. *Niphargus* Schiødte, 1849 (Niphargidae) is the most diverse genus of freshwater amphipods, comprising hundreds of narrowly endemic species distributed across West Palearctic groundwaters. Nevertheless, a handful of species are known to occur in surface waters as well, and two

of these, *N. hrabei* and *N. valachicus*, have extremely large ranges (>1300 km) that even exceed those of many surface amphipod species. We hypothesize that these two species were able to secondarily colonize the epigean environment and that this ecological shift facilitated their large-scale dispersal. Phylogenetic analyses comprising almost half of the known taxa of *Niphargus* revealed that *N. hrabei* and *N. valachicus* are not closely related, despite their morphological, ecological and biogeographic similarities. Moreover, ancestral state reconstruction strongly indicates that both species have independently colonized the epigean realm. Mitochondrial

and nuclear data strongly support the conspecificity of geographically distant populations, although they exhibit significant genetic differentiation. Molecular dating and Bayesian diffusion models suggest Late Pleistocene dispersal from the Wallachian Plain for *N. hrabei* and Middle Pleistocene dispersal from the SW Pannonian Basin for *N. valachicus*. For the latter, the Pannonian Basin apparently served as a long term glacial refugium, corroborating previous evidence from other aquatic taxa. The Danube River

and its major tributaries served as important corridors for dispersal. Morphological analyses revealed that these two species are closer to each other than they are to most other epigean *Niphargus* species, and were clustered together with small bodied, interstitial species. In conclusion, our results indicate for the first time that adaptation to groundwater is not a one-way evolutionary path and that groundwater species can occasionally recolonize and widely disperse in surface waters.

Epigean gammarids survived millions of years of severe climatic fluctuations in high latitude refugia throughout the Western Carpathians

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ABSTRACT

Due to its glacial refugium role during the Pleistocene glaciations, the peri-Mediterranean area harbours a greater diversity than northern latitudes. Although extra-Mediterranean refugia were identified in Western Carpathian terrestrial species, evidence for epigean aquatic taxa remained elusive. We compared the geographical patterns of lineage diversity of the *Gammarus fossarum* species complex between the Bohemian Massif and the Western Carpathians. Our results revealed the presence of eight lineages of Miocene age: six are relict and endemic to the Western Carpathians while the remaining two exhibit demographic expansion and inhabit both regions. Their distribution does not seem to be significantly constrained by river catchments or topography. Thus, the observed patterns of diversity likely result from historical rather than contemporaneous factors. Despite the high latitude, the Western Carpathians functioned as a long term glacial refugium for permanent freshwater fauna, facilitating the survival of ancient lineages through millions of years of severe climatic fluctuations.

KEY WORDS

Biogeographical barrier; cryptic diversity; endemism; *Gammarus fossarum*; northern refugia.

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Pleistocene glacial cycles greatly influenced the present-day patterns of biodiversity across Europe. Southern peninsulas served as glacial refugia and therefore may harbour significantly higher diversity than the northern, formerly glaciated areas. Nevertheless, northern glacial refugia (outside the Mediterranean area) have been documented in Central Europe for a number of terrestrial taxa, but conclusive evidence for epigean aquatic species has remained elusive. In this study, we focused on the widespread epigean *Gammarus fossarum* species complex. Using mitochondrial and nuclear markers, we compared the geographical patterns of lineage diversity of this species complex between the Bohemian Massif and the Western Carpathians, two

adjacent Central European regions which exhibit distinct biogeography and geomorphology. We explored whether the observed spatial patterns of genetic diversity are more likely to result from historical or present-day factors. Phylogenetic and phylogeographic analyses revealed eight phylogenetically diverse lineages. Two of them display local signatures of recent demographic expansion and inhabit both regions. The remaining six lineages exhibit a relict distributional pattern and are found only in the Western Carpathians. Molecular dating indicates that these lineages are old and probably diverged throughout the Miocene (7 to 18 Ma). Furthermore, their distribution does not seem to be constrained by the present boundaries of river

catchments or topography, as they are present in adjacent drainages and have overlapping altitudinal ranges. Thus, the contrasting spatial patterns of diversity observed between the two regions more likely result from historical rather than contemporaneous or recent factors. Specifically, our molecular results are in good agreement with the fossil record that small, isolated patches of deciduous forests have survived glacial episodes across the Western Carpathians, but probably not in the

Bohemian Massif. We propose that the rugged terrain and the existence of stable, deep circulation mineral springs in the former region facilitated the persistence of gammarids. Our results indicate that, despite the high latitude and proximity to Pleistocene ice sheets, the Western Carpathians functioned as a long term glacial refugium for permanent freshwater fauna, allowing the uninterrupted survival of ancient lineages through millions of years of severe climatic fluctuations.

Importance of systematic in knowledge and protection of biodiversity, the case of the genus *Haploops* Liljeborg, 1856 (Ampeliscidae)

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ABSTRACT

A complete revision of the *Haploops* Liljeborg, 1856 (Ampeliscidae) species is in course with important collections coming from the North Atlantic mainly during the BIOICE and BIOFAR programmes. Till today, *Haploops* accounted 23 species; several species remained to describe. We propose a review of the available data on this genus including taxonomy, biogeography, ecology and biology and some ways of research in the future on this genus.

KEY WORDS

Haploops; Ampeliscidae; systematic; ecology; biodiversity.

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Since the overview of the genus *Haploops* Liljeborg, 1856 (Ampeliscidae) at the end of the 1980's (Dauvin & Bellan-Santini, 1990), several new species of this genus had been described in the Atlantic Ocean (see Kaim-Malka et al., 2016 and reference therein). So, the number of species have increased from 15 at the end of the 1980s to 23 nowadays. New records were mainly acquired during the BIOICE and BIOFAR programmes for the North Atlantic Ocean or the Bay of Biscay. With 15 species the North Atlantic Ocean is the richest (Fig. 1). Several species were re-described and other new species remained to be describe.

Moreover, some recent ecological studies mainly from the North-eastern Atlantic (Rigolet et al., 2011, 2012, 2014a,b; Koop et al., 2013; Dubois et al., 2015) and in the North-Atlantic and Arctic Ocean focused on the role of the *Haploops* high density as an 'ecosystem engineer' in the macrobenthic communities functioning (Highsmith &

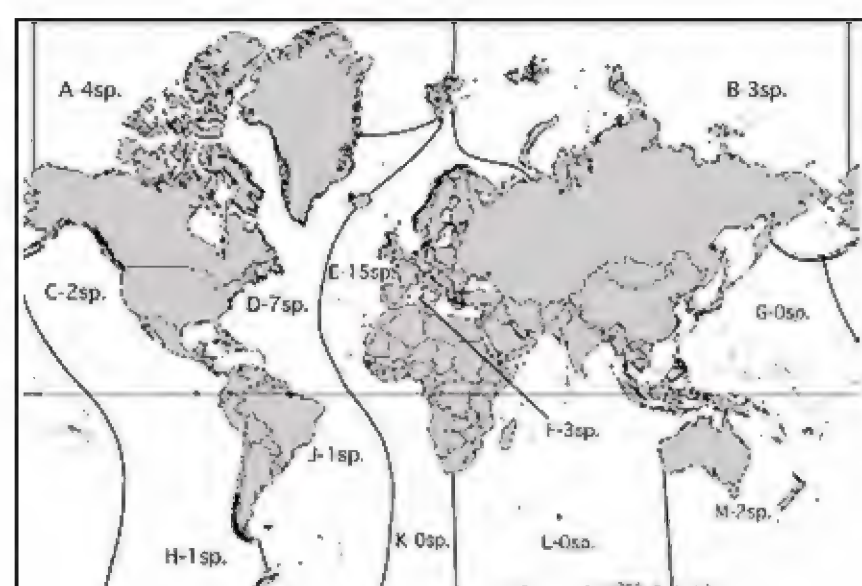


Figure 1. Geographical distribution of the 23 species of *Haploops* from the world's Ocean: *H. abyssorum* (E); *H. antarctica* (H); *H. antennata* (E); *H. carinata* (D, E); *H. dellavallei* (F); *H. descansa* (M); *H. fundiensis* (D); *H. gas-cogni* (E); *H. islandica* (D, E); *H. laevis* (A, B); *H. longiseta* (E); *H. lodo* (C, E); *H. nirae* (F, E); *H. oonah* (M); *H. proxima* (F, E); *H. robusta* (E); *H. setosa* (A, D, E); *H. sibirica* (A, B); *H. similis* (D, E); *H. tenuis* (E); *H. tubicola* (A, B, C, D, E); *H. vallifera* (D, E); *H. meloi* (J).

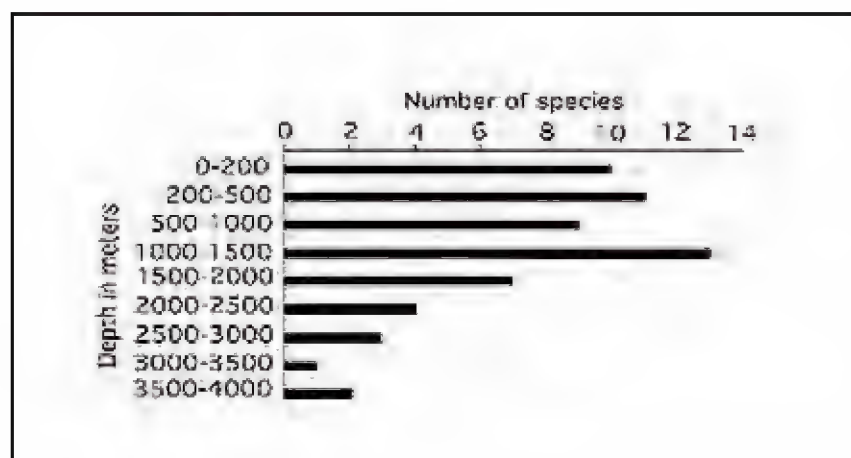


Figure 2. Samples depth range of the 23 species of *Haploops* from the world's Ocean. Nine species were only recorded in a single depth range: 3 (0–200 m); 2 (500–1000 m); 3 (1000–1500 m); 1 (3500–4000 m).

Coyle, 1992; Shields & Hughes, 2009; Conlan et al., 2013). Secondary production and relation with the pockmarks are studied in the north of the Bay of Biscay which show high link between the dense populations of the tubicolous *H. nira* and the formation and the colonisation of the pockmarks (Rigolet et al., 2014; Dubois et al., 2015).

New biogeographical data concern also deep-sea species (Bellan-Santini & Dauvin, 2008; Barry et al., 2013). *Haploops* are found from shallow water to 3,800 m (Fig. 2). In this note, we present new available information on the genus *Haploops* since the Dauvin & Bellan-Santini (1990) overview, on the taxonomy, biogeography, ecology and biology and propose some ways of research in the future. We emphasize the importance of a high quality taxonomy process, based on precise descriptions, accompanied by topographical and ecological data, as accurate as possible. These data may be of major efficiency in the context of a reasonable estimate of the biodiversity temporal changes and the need to preserve the marine ecosystems.

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Amphipod importance in the trophic network of the eastern part of the English Channel

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ABSTRACT

Benthic and suprabenthic macrofauna and demersal before fish has been sampled on a future offshore wind farm site in the eastern English Channel. The amphipods are among the dominant group of the macrofauna and dominate the suprabenthos and fish stomach content. Seasonal and spatial differences between two benthic habitats (sand and gravel) occur showing the higher importance of amphipods on sand than on gravel in spite of a higher diversity and abundance on gravel. The trophic transfers between benthos, suprabenthos and fish are discussed.

KEY WORDS

English Channel; stomach contents; amphipods in trophic chain.

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The French government has set a target of 23% of energetic consumption derived from renewable energy sources including Marine Renewable Energy for 2020. Eight Offshore Wind Farms (OWF) are planned and among them Dieppe-Le Tréport site in the eastern English Channel (eEC). In order to further knowledge on the structure and functioning of the ecosystem before the installation of OWF, the PhD thesis of J.P. Pezy is focused on the establishment of the trophic network within ECOPATH model (Pezy et al., 2017) on this site in comparison with similar benthic habitats from the eEC. The collection of new data on biological compartments (zooplankton, suprabenthos, meiofauna, benthos and demersal fishes) are essential. So, four campaigns (summers 2014 and 2015 then winters 2015 and 2016) has permitted to estimate the contribution of each zoological group in two main benthic habitats: i.e. sandy gravels and medium clean sands, founded on the site (about 30 km²)

(Fig. 1). Macrofauna was sampled with a 0.1 m² Van Veen grab (five replicates in 25 stations), suprabenthos with a modified Macer-Giroq sledge (Dauvin et al., 1994) in one station from each benthic habitat (day and night sampling) and fish with a beam trawl in ten stations including four on the sand habitat and six in the gravel habitat. A total of 1,584 demersal fishes has been collected and their stomach contents has been examined. The data are grouped in two seasons (summer and winter) and two main benthic habitats sandy gravel (19 stations) and medium sand (6 stations) has been identified. A total of 42 amphipod species has been recorded: 37 on gravel, 25 on sand for the benthos, 26 in the suprabenthic samples and 24 in the stomach contents.

There are high seasonal and spatial difference between both habitats, with a higher proportion of amphipods in summer than in winter to the sandy gravel habitat than medium sand habitat for the

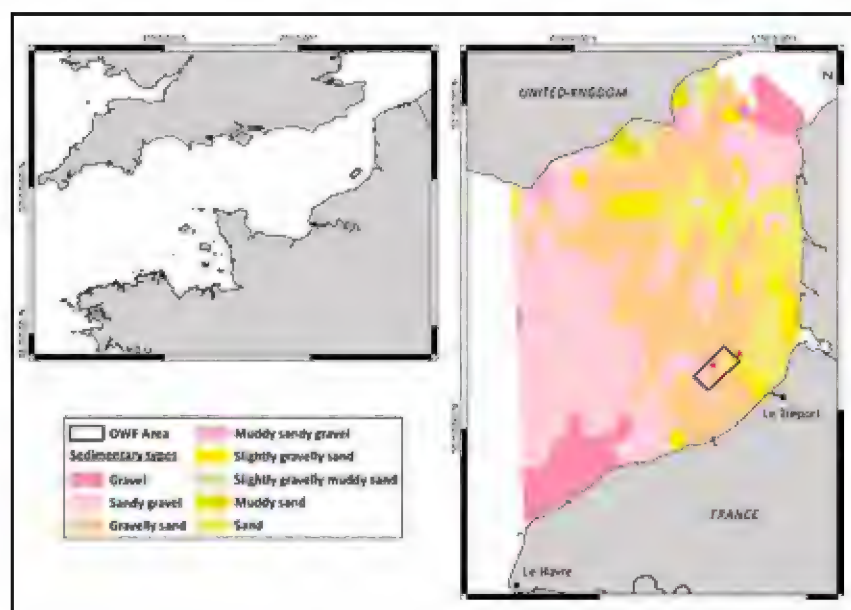


Figure 1. Location of the sampling zone in the eastern part of the English Channel. Red star corresponds to the suprabenthic samples in both medium sand and sandy gravel habitats.

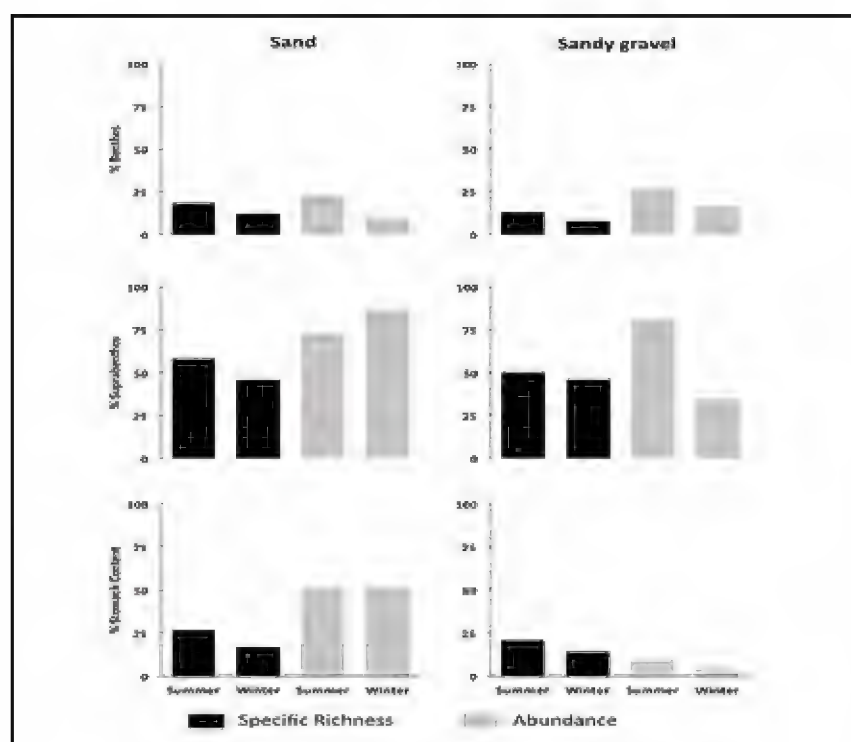


Figure 2. Proportion of amphipods in the benthos, suprabenthos and stomach contents for both seasons and both habitats in the Dieppe-Le Tréport area.

benthos, while the suprabenthos showed higher proportion on medium sand than on sandy gravel. Similarly the amphipods formed >51% of the preys on fish sampling on medium sand and less than 10% of the fish sampling on sandy gravel. It appeared that the amphipods are much consum-

mated while the large bivalves which form the main part of the benthic biomass are unconsumed. Thus it exists a disparity between the assessment of benthic biomass and prey looked by the fishes.

The stomach content results obtained in this part of the English Channel are compared with those obtained in the Bay of Seine (Mallek-Zouhiri, 1999; Tous Rius, 2009) and the Bay of Morlaix (Dauvin, 1988). The role of the amphipods in the fish prey item through their accessibility in the suprabenthic layer are underlined. Moreover, high differences between both benthic habitats underline the fundamental role of surficial sediment at small spatial scale and their specific assemblages in the trophic food web.

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Microsporidia infecting gammarids: ancient association or recent acquisition following host spread?

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ABSTRACT

Numerous species of microsporidian parasites are known to infect gammarids, but the specificity of infection and the co-evolution between these hosts and these parasites remain overlooked. The evolutionary history of *Gammarus balcanicus* Schäferna, 1922 and *G. roeselii* Gervais, 1835 (Gammaridae) throughout Europe has just recently been solved. We took this opportunity to study the patterns of microsporidian infection in these two morphospecies, with the aim to answer the following specific questions. First, given that *G. roeselii* diversified within the Balkan area and recently spread to Northern and Western Europe, did the host and the parasite co-evolved within the Balkans? Was the vertical transmission (VT) from female to progeny a key factor controlling co-evolution? Conversely, did *G. roeselii* recently acquired parasites from the local host species within its North-Western area of expansion?

KEY WORDS

Gammarus roeselii; *G. balcanicus*; microsporidia; biogeography; co-evolution.

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INTRODUCTION

Microsporidia are obligatory endosymbiotic eukaryotic microorganisms that can be found in many animal hosts. Two strategies of transmission have been observed in microsporidia to infect their hosts: horizontal transmission (HT) from an individual host to another by contagion and vertical transmission (VT) from female to its progeny. During the last twenty years, numerous microsporidian infections have been detected in freshwater amphipods, especially in the genus *Gammarus* Fabricius, 1775 (Gammaridae) (Bacela-Spychalska et al., 2012; Ironside & Alexander, 2015; Grabner et al., 2015; Terry et al., 2004), where the VT transmission strategy is found to be common (Terry et al., 2004; Haine et al., 2007; Hogg et al., 2002). In gammar-

ids, the pattern of infection specificity remains unclear: a single parasite species (or group of closely-related species) can infect several host species and, conversely, a single host species can be infected by several parasite species (e.g. Grabner et al., 2015). This pattern does not fit the hypothesis that VT favors the co-evolution between parasites and hosts but, instead, suggests frequent horizontal transfers and parasite adaptation to different hosts. However, this hypothesis has never been rigorously tested. The evolutionary history of *Gammarus balcanicus* Schäferna, 1922 and *G. roeselii* Gervais, 1835 in Europe have recently been solved. Both morphospecies are characterized by very high local endemism and ancient diversification (ca 15MY) in south-east Europe (Mamos et al., 2016; Grabowski et al., 2017). The aim of the present study is to investigate

microsporidian infections in the whole geographical range of these two host morphospecies, to explore if parasites have co-evolved with their host thanks to VT, or if they have been acquired recently by HT. *G. roeselii* is particularly interesting to this respect, since this species have spread in North-Western Europe, where it came into contact with several local species known to harbor microsporidia (Terry et al., 2004). Moreover, *G. roeselii* is known to be infected by microsporidia both found in other gammarid species or apparently more specific (e.g. Haine et al., 2007). Therefore, the diversity of microsporidia in each host species will be established and the evolutionary history of microsporidia will be assessed.

MATERIAL AND METHODS

Gammarids were collected in the field from 2004 to 2016, fixed in 99% ethanol, and stored at the Department of Invertebrate Zoology & Hydrobiology, University of Lodz. The total DNA was extracted using standard Phenol/Chlorophorm method, or using QIAGEN DNeasy® kits from 1253 individuals of *G. balcanicus* from 116 sites as well from 1119 individuals of *G. roeselii* from 103 sites.

PCR screening for microsporidia were performed using short specific primers: V1F (5'-CAC CAG GTT GAT TCT GCC TGA C-3', see Terry et al 2004) and UNIR (5'-TCA GGC TCC CTC TCC GGA AT-3', R. Wattier, unpublished).

For the purpose of phylogeny reconstruction we used primers V1F and HG4R (5'-GCGGCTTA-ATTGACTCAAC-3', K. Bacela-Spychalska, unpublished), generating 950 bp-long sequence, or V1F and 530R (5'-CCG CGG CTG CTG GCA C-3') generating a 500 bp-long sequence.

RESULTS AND DISCUSSION

We found a wide distribution of microsporidia infection in both species of gammarids all over their geographic range (Fig. 1). The prevalence (proportion of infected individuals) was ca. 30% in *G. balcanicus* and ca. 20% in *G. roeselii*. There was nevertheless a high variation among sites, with some populations being heavily infected and some

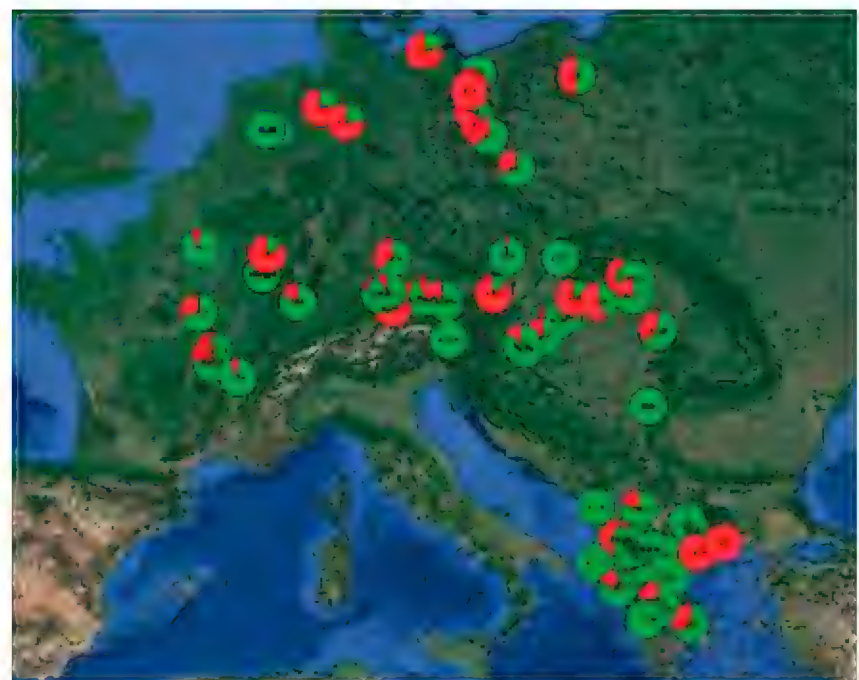


Figure 1. Microsporidia prevalence for *G. roeselii* in Europe. Each circle represent a single site. Green: proportion of uninfected individuals; Red: proportion of infected individuals.

being uninfected. The populations in the Balkans, where both host species diversified, were infected, but the infection was significantly lower in this region than outside the Balkans (e.g. for *G. roeselii*: Pearson's Chi-squared test; p-value = 0.001). The diversity of microsporidian parasites found in the infected populations will be discussed and confronted to the biogeography of their hosts.

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Gammarids, acanthocephalan parasites and temperature: potential effects on trophic interactions

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ABSTRACT

In a series of experiments, we investigated the interaction between the infection by acanthocephalan parasites and temperature variations on various behaviour of *Gammarus fossarum* Koch, 1836 (Gammaridae).

KEY WORDS

Gammarus fossarum; *Pomphorhynchus terreticollis*; temperature variation; behaviour.

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INTRODUCTION

Acanthocephalans parasites are well known to disturb behavior of gammarids in various ways. In particular, they influence the probability to be eaten by fish or birds (either increasing or decreasing predation risk). They also modulate the way gammarids feed. These parasites therefore have a strong potential effect on the ecological role of these crustaceans, either as shredders or preys, in rivers or streams.

A number of studies have also attracted the attention on the role of global warming on the ecological role of keystone species. For gammarids, it is well known that temperature modulates the feeding rate and/or the quality of food ingested.

To our knowledge, the investigations upon the effects of the interaction between parasitism and temperature variation on the ecological role of gammarids are lacking, as well as the effect of this interaction on gammarids behavior.

RESULTS AND CONCLUSIONS

By investigating gammarids naturally infected

by acanthocephalan parasites under different temperatures, we showed that (i) increased temperatures per se induced a significant increase in consumption of dead leaves (shredding role of gammarids), but only to a certain extent. Infection by *Pomphorhynchus terreticollis* (Rudolphi, 1809) significantly negatively impacted the shredding role of *Gammarus fossarum* Koch, 1836 (Gammaridae).

However, consumption at the highest temperature depended on amphipod density: Whereas a decrease was observed for single individuals, no such effect on feeding was observed for individuals in groups (Labaude et al., 2017a); (ii) the phototaxis of *G. fossarum* increased with increasing temperature, with a stronger effect in gammarids infected by *P. tereticollis*. This is the first evidence that temperature can affect the extent of behavioral alteration induced by certain parasite species. In contrast, temperature had no effect on the alteration of refuge use by the infected gammarids or on their geotaxis (Labaude et al., 2017b).

Therefore, in terms of increased trophic transmission, the consequences remain elusive, as the key anti-predatory behavior was not significantly

affected by exposure of gammarids to different temperatures.

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Population traits of *Atlantorchestoidea brasiliensis* (Dana, 1853) (Talitridae) along the coastline of Uruguay: combined information from demography, behaviour and Cytochrome Oxidase I

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ABSTRACT

Talitrid populations from beaches along the Rio de la Plata estuary were described in terms of traits detectable at local scale.

KEY WORDS

Sandy beaches; salinity; *Atlantorchestidea brasiliensis*; behaviour; population.

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The coastline of Uruguay, extending from the Rio de la Plata estuary to oceanic shores, represents an optimal natural setting to test the effects of salinity variation. In particular, when targeting resident supralittoral fauna, the consideration of population level allows for the identification of matches between population and the local environment (Begon et al., 2006). Across salinity conditions naturally ranging from 4.5 to 32.3 psu, we then analysed population traits of the supralittoral talitrid *Atlantorchestoidea brasiliensis* (Dana, 1853). Three sites located at different points along the coastline of Uruguay were selected: the inner site (“Baguala”) is the estuarine limit where resident populations of *A. brasiliensis* can be found (Lercari & Defeo, 2015). The sites “Arachania” and “Barra del Chuy” are considered oceanic as they are located beyond the limit of the estuary (after Martínez & Ortega, 2015). Resident talitrid populations were analysed for life-history (abundance, sex ratio and

juvenile ratio), substrate choice (preference for high salinity) and Cytochrome Oxidase I (COI) divergence. Distance (in km) from the site closest to the estuary was used as discriminant for the tests, namely: repeated measures ANalysis Of VAriance (ANOVA) for demographic traits; chi-square test with Yates'correction for substrate choice with inter-rater agreement estimated with Cohen's coefficient k ; Analysis of MOlecular VAriance (AMOVA) for population structure based on COI sequences. Furthermore, by sequencing the same individuals tested for behaviour, it was possible to pair the data and test with AMOVA the two groups expressing low and high substrate salinity choice.

Abundance of *A. brasiliensis* decreased with distance from the estuary. The proportion of females also decreased significantly towards high salinities, while the juvenile ratio did not vary significantly among sites. The test for binary choice on substrate salinity preference revealed behavioural differences

among populations: the population closer to the estuary showed no substrate preference, while individuals from the other two sites significantly preferred high salinity substrates. The lack of preference was expressed at individual level, i.e. without agreement between choices of the same individual in subsequent days. COI sequence analysis showed the population from the intermediate site to differ significantly from the other two, while no significant genetic differentiation was found between populations from the two most distant sites, nor between individuals that expressed different salinity preference. Results 1) confirmed the reproductive strategies identified by Gómez et al. (2013) triggered by optimal salinity condition and 2) showed that diverse sets of traits at the population

level enable sandy beach species to cope with local environmental changes: life-history and behavioural traits appear to change in response to different ecological conditions. On the other hand, demographic and behavioural traits seemed to be independent of the population structure inferred from COI sequence variation. Population profiles characteristic of each site were consequently identified (Figure 1). Results pointed to the relevance of spatial scale to capture both the effects of movements from/to neighbouring populations along a macroarea, and the local expression of traits.

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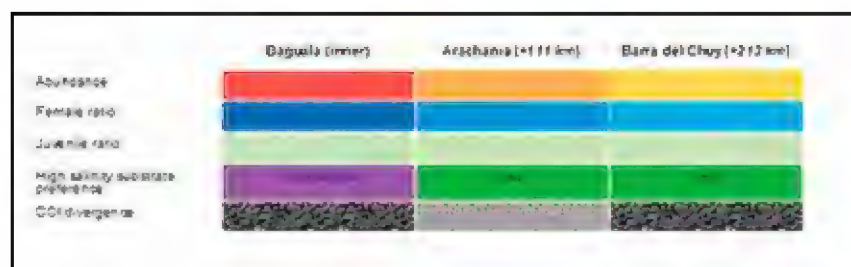


Figure 1. Population profiles as resulted from the set of traits analysed. Distance from the inner estuarine site is reported in brackets beside the toponyms. Within a line, corresponding to a trait analysed, different colors or patterns indicate significant differences between populations. Gradients of the same color indicate significant increasing/decreasing values. Same color and shade indicates no significant variation among populations.

A combined biological and social approach to test the role of recreational boating as vector for exotics: the case of peracarids in the Mediterranean Sea

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ABSTRACT

Shipping is known to be a major vector for introduction and spread of marine non-indigenous species (NIS), however, biofouling remains unregulated and fouling on recreational boats has been overlooked. This is the first large-scale study exploring the role recreational boating plays in NIS transport across the Mediterranean Sea, focusing on Peracarida. A combined biological and social approach was used by analyzing the community from boat hulls and marina docks across the Mediterranean; and by conducting interviews with boat owners. A surprisingly high number of alien peracarids were found on vessels cruising Mediterranean waters. No geographical gradient across the Mediterranean was found regarding vessel fouling composition; local conditions of each marina played a bigger role. Boats with lenient cleaning habits which undertake long journeys were more susceptible to host peracarids. This study proves that recreational boating is a high-risk vector in the Mediterranean Sea, and that there is a lack of awareness among boaters, which calls for environmental education strategies.

KEY WORDS

Non-indigenous species; Peracarida; recreational boating; Mediterranean Sea; maintenance habits.

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INTRODUCTION

In marine ecosystems, shipping is known to be a main vector for both the primary introduction and the secondary spread of non-indigenous species (hereafter NIS), via ballast water or biofouling (Ruiz et al., 2000). While an agreement on the management of ballast-water vector has been reached (IMO, 2017), biofouling is not yet managed, and fouling on recreational boats in particular has been overlooked (Minchin et al., 2006). At present, only New Zealand, Australia and Canada are following initiatives at national level for regulating fouling

treatment; elsewhere, recreational boating remains an unregulated vector, and management relies at best on voluntary initiatives (International Maritime Organization, IMO, 2012). The Mediterranean Sea alone attracts 70% of the global recreational boat activity but the efficacy of recreational boating in transporting NIS here has yet to be assessed. This is the first large-scale study exploring the role recreational boating plays in the spread of NIS across the Mediterranean Sea, focusing on peracarids, particularly amphipods and isopods as model taxa. This work presents a unique combined biological and social approach: (1) personal interviews

with boaters to understand their boat characteristics, maintenance habits travel history and awareness of the issue, and (2) marina and boat hull sampling to determine the fouling species assemblage. The two aims of this research are to explore the distribution patterns of native versus non-indigenous peracarid species in artificial habitats across the Mediterranean (both boat hulls and marinas), determine if recreational boating is an effective vector in the transfer of non-indigenous peracarids, and investigate which maintenance habits of boat owners predispose the occurrence of biofouling on boat hulls.

MATERIAL AND METHODS

Between April and October 2015 fouling assemblages were collected from boat hulls and marina docks from 10 recreational marinas across the Mediterranean Sea. After identification, each species was firstly classified as 'native', 'NIS' or 'cryptogenic', and secondly as 'potential invader' versus 'native with a restricted distribution', in order to assess the influence of the recreational boating vector under a global perspective. Peracarid community composition on hulls was analyzed through a two-way PERMANOVA design testing the factors 'subregion' of the Mediterranean Sea and 'marina'. Several parameters including NIS/total ratio and potential invader/total ratio were calculated. Then, the relationship among the behavioural aspects and the occurrence of fouling Peracarida was explored using multiple linear regression analysis.

RESULTS AND DISCUSSION

A total of 247 interviews were conducted, and the same number of boat hulls were inspected from 10 different Mediterranean marinas. Our results reveal that recreational boats cruising Mediterranean waters host a surprisingly high number of non-indigenous peracarids, sometimes transporting them to new marinas, from where they may subsequently colonize the hulls of berthed boats. We found that 64% of the analyzed boats carried peracarids; from those 56% carried NIS and 79% carried exclusively potential invaders in their hulls. No geographical gradient across the Mediterranean was found regarding Peracarida composition on

vessels (no significant effect of 'subregion'); but rather, local conditions of each 'marina' played a more important role ($P(\text{MC})_{\text{marina}}=0.0001$). In fact, similar fouling compositions were observed on vessels sampled at opposite subregions of the Mediterranean Sea. The travel history of boaters may have affected marina community composition over time, making each marina unique, and in turn influencing the fouling composition of adjacent vessels. Moreover, fouled boats continuously connect marinas, regardless of distances that would normally inhibit the spreading of peracarids. The resulting scenario is an expansion of non-indigenous amphipods and isopods that may eventually lead to biotic homogenization (Olden & Poff, 2004; Galil, 2007). Finally, the multiple regression analysis revealed a significant model ($F(3, 49) = 6.798$, $P = 0.0006$, $n = 53$, $R^2 = 0.29$) and suggested that boats with lenient cleaning habits (once per year or less) that undertake long journeys (> 400 miles) were more susceptible to host peracarids (time since last cleaning, $P = 0.012$; $\beta = 0.03$; longest distance travelled, $P = 0.023$ $\beta = 0.0003$). Although both cleaning practices and places visited are listed as influencing factors by IMO (2012), most of the boaters were found to perform insufficient cleaning and when interviewed, they were unaware their boat could carry NIS or claimed it to be perfectly clean despite the presence of several taxa.

This study provides evidence that recreational boating is a high-risk vector for transport of non-indigenous amphipods and isopods in the Mediterranean Sea, and this should warrant regulation. In addition, these results demonstrate a lack of awareness among boat owners which should be addressed with environmental education strategies

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The antennae and the astronomical orientation of *Talitrus saltator* (Montagu, 1808) (Crustacea Amphipoda)

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ABSTRACT

To orientate along the sea-land axis of the beach the sandhopper *Talitrus saltator* (Montagu, 1808) (Crustacea Amphipoda) can rely on both the sun and the moon. There are evidences that separate chronometric mechanisms are accountable for the compensation of the daily azimuthal variations of the two celestial bodies. In this work we demonstrate that the antennae are the putative anatomical site of the moon compass mechanism since they are essential for a proper lunar orientation whereas the ability to orientate to the sun is not compromised by the removal of these appendages.

KEY WORDS

Sandhopper; *Talitrus saltator*; antennae; astronomical orientation.

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The supralittoral sandhopper *Talitrus saltator* (Montagu, 1808) is known to rely on several orienting factors to return to the belt of damp sand of the beach, in which live buried, following the shortest route (the sea-land axis of the shore) and thus minimizing the effects of environmental stressors. Both sun and moon constitute important astronomical cues in the zonal recovery of this species. Their use requires time-compensatory systems to account for their daily azimuthal variations and it seems that in *T. saltator* the sun and the moon compasses are regulated by separate chronometric mechanisms (Ugolini et al., 1999). The same time-keeper involved in the functioning of the sun compass also sustains the circadian rhythm of locomotory activity exhibited by this species (Ugolini et al., 2007).

Chronometric mechanisms located in the antennae, accountable for the compensation of the daily apparent movements of the celestial bodies, have been demonstrated in the Monarch butterfly *Danaus*

plexippus (Merlin et al., 2009) and in *T. saltator* (Ugolini et al., 2016).

In this work we illustrate our recent progresses in the knowledge of the astronomical orientation in *T. saltator* as regards the role of the antennae in the proper functioning of the time-keepers.

We conducted both behavioural and molecular investigations.

Tests of solar and lunar orientation of: 1) intact animals, 2) animals with first and/or second antennae ablated and 3) animals with right (both first and second) antennae painted with black enamel were conducted. Experiments were carried out in a confined environment with the vision of the natural sky and sun/moon. Data were elaborated according to methods proposed by Batschelet for circular distributions.

To establish the eventual impairment of the mechanism regulating the sun compass, the locomotor activity of intact and “antennaeless” individuals

(subjected to a L:D = 12:12 cycle in phase with the natural photoperiod) was also recorded for at least 10 days using a micro-wave radar system. Molecular analyses were focused on the evaluation of rhythmicity in the cyclic expression of four circadian clock genes (*Per*, *Clk*, *Tim* and *Cry2*) in both brain and antennae. Total RNA of individuals entrained under an L:D = 12:12 cycle followed by a 24 h free-running period in DD, was extracted from either tissues, reverse-transcribed and subjected to Taqman qPCR. Tissues samples were harvested at three hours-intervals over a 24 h period. Rhythmicity in gene expression was evaluated using Cosinor software.

Solar orientation tests show that ablation and unilateral painting of the antennae do not affect the chronometric mechanism of sun compass. Individuals lacking both first and/or second antennae or with right antennae dark-painted exhibit a mean orientation, as intact animals, in good agreement with the expected direction. Moreover, even the circadian locomotory rhythm is not disrupted by the removal of these appendages. Instead, when tested under the moon sandhoppers with ablated (first and/or second) and unilaterally painted antennae show a positive phototactic response to the moon while intact individuals are well orientated toward the expected directions.

Molecular investigations reveal statistically significant oscillations in the circadian expression of the genes *Per* and *Cry2* in both tissue.

In conclusion, our results demonstrate that in *T. saltator* the antennae have competence to time-

keeping and are necessary to properly orientate to the moon. They confirm previous works on the existence of discrete time-compensatory mechanisms regulating the sun and the moon compasses in this species: indeed they seem located in separate anatomical districts, the brain and the antennae respectively. However, the disruption of the lunar orientation ability in individuals with unilaterally dark-painted antennae suggests a possible downstream integration of timing information from right and left antennae in the brain, as demonstrated in *D. plexippus*. Further investigations are required to clarify this aspect.

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The family Niphargidae in Central and Western Europe

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ABSTRACT A project at the Université libre de Bruxelles deals with molecular taxonomy of Niphargidae in Luxembourg, France, Belgium, the Netherlands, and Central Europe

KEY WORDS Niphargidae; *Niphargus*; molecular taxonomy; Western Europe; Central Europe.

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The family Niphargidae inhabits mainly ground-water and is distributed all over Europe and the Middle East. The number of species of niphargids in Western and Central Europe is uncertain: several morphological characters which were used to describe the species have proven to be variable, while others are misleading due to convergence or parallel evolution. These facts suggest that older descriptions and determinations are strongly questionable. Moreover, it is assumed that many cryptic species remain to be discovered.

Preliminary results from a project carried out at the Université libre de Bruxelles dealing with molecular taxonomy of Niphargidae in Luxembourg, France, Belgium, the Netherlands, and Central Europe have been reported. The main aim of the project is to compare the results of molecular and morphological methods for an integrative taxonomy of the niphargids in the selected area, estimate species richness, discover possible cryptic species, as well as identify distributional patterns analysing the effects of the last Quaternary glaciation on both species richness and distribution.

As older collections are either lost or preserved in 70% ethanol, they are not suitable for sequencing. Hence, all specimens had to be re-collected.

Collections were done in caves and mines (hand collection, tin can baits), springs (sieve), wells (plankton net), and interstitial (Bou-Rouch pump). The collected specimens were morphologically sorted (to avoid the wording “determined”).

From each specimen, two independent markers were PCR-amplified and then sequenced: the Folmer fragment of the mitochondrial COI gene and a fragment of the nuclear 28S ribosomal gene. Chromatograms were assembled into contigs and cleaned using the program Sequencher. Molecular phylogenies were obtained for each marker using the software MEGA6. The trees were then compared in a tanglegram to identify congruent and incongruent parts of the trees. If some incongruent areas were identified, they were investigated in detail using other markers such as the ITS region and other independent nuclear markers.

Preliminary results are promising. *Niphargus puteanus* is so far the only species where identification keys based on morphology and analysis of sequences give unequivocal results.

As regards *Niphargus schellenbergi*, 55 specimens from 41 sites in France, Luxembourg, and Germany were sequenced, indicating that *N. schellenbergi* is a good species. However, in about 5%

of the cases, determination keys failed: we found *N. schellenbergi* with rounded outer edges of the 3rd epimeral plate (where so far the 90° angle counted as a clear identification character) and we found *N. schellenbergi* with 5 setae on the 2nd dactylus of the gnathopodes (where 2-4 are characteristic for *N. schellenbergi* while 5 indicate *N. puteanus*).

Niphargus virei was found for the first time in Germany. Surprisingly, the German specimen is closer to the phyletic line from Ardèche than to the one distributed in Belgium and the Netherlands. *N. virei* is well known to be a complex of at least three cryptic species.

Finally, *Niphargus aquilex*, *N. rhenorhodanensis*, *N. thienemanni* and *N. fontanus* need more specimens to be sequenced to get unequivocal result; comparing our data with sequences retrieved from GenBank, cryptic species are concealed under the names *N. aquilex*, *N. rhenorhodanensis* and maybe *N. fontanus* in the study area.

First results show that previous publications from the research area based only on morphological features have to be verified. It is expected that, by

end of the project, the number of species in the selected area will double.

Although about 3000 specimens from 256 sites were collected so far, many gaps remain in the study area. In case someone wishes to help with collecting or have already collected specimens preserved in concentrated, non denatured ethanol, please contact the authors.

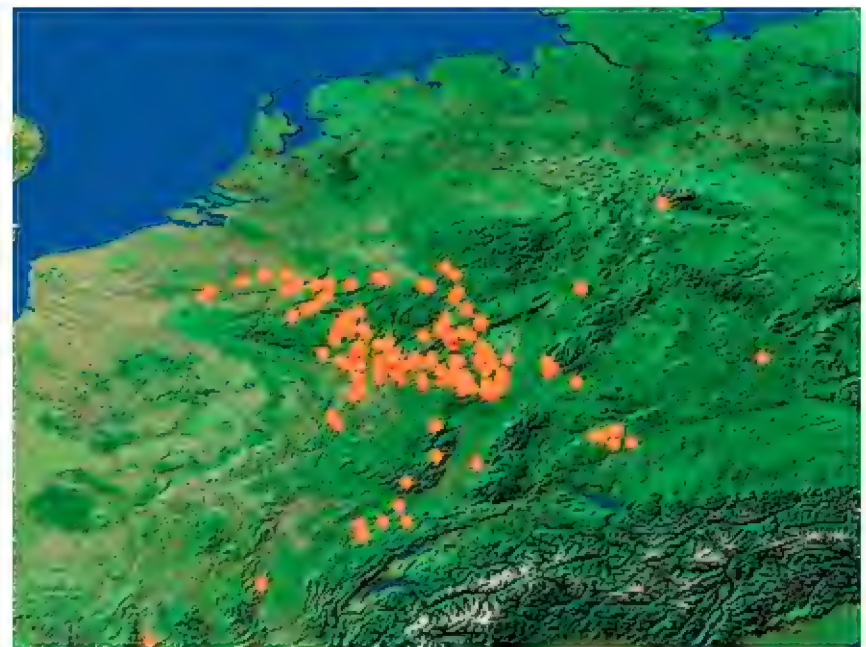


Figure 1. Map of the research area and sampling sites until March 2017.

Functional importance of freshwater amphipods in the leaf litter recycling process: the role of leaf litter characteristics

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ABSTRACT Impact of leaf characteristics on the keystone amphipod species for litter decomposition process.

KEY WORDS Terrestrial inputs; riparian trees; biomechanical structures; chemical composition.

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Leaf litter decomposition is an essential ecosystem function that contributes to carbon and nutrient cycling in freshwater streams and terrestrial inputs provide an essential energy source for many freshwater organisms. Processing of leaf litter is complex and involves several physicochemical (e.g. hydrologic fractioning, water chemistry, leaf litter characteristics) and biological (e.g. the conditioning process by aquatic fungi, the biomass of shredder) factors. Even if the decomposition process involve both physical and biological factors, the role play by shredder macroinvertebrates remains quantitatively essential. Among shredder invertebrate species, amphipods appears as a key assemblage for the leaf litter breakdown processes in streams due to their high abundances and the very high feeding rate (Piscart et al., 2009, 2011).

In order to disentangle the role of both the leaf characteristics and conditioning process by aquatic hyphomycetes, we performed several field and experimental studies using freshwater gammarid species and different types of leaves. In a first experiment in lab condition, we compared the shredding efficiency of 12 species of amphipods

and one isopod with alder (*Alnus glutinosa*) leaves in order to highlight the between- species variability. The study highlighted a very great variability in the relative consumption rate of shredders varying from 0.09 to 0.64 gram of leaf per gram of shredder per day.

In a second experiment (Foucreau et al., 2013), we used the very common freshwater amphipod *Gammarus pulex* (Linnaeus, 1758) (Gammaridae) as shredder species and five types of leaves differing in toughness: alder leaves which are very common along streams and rivers and are considered as soft and readily consumed by aquatic invertebrates; and two hornbeams (*Carpinus betulus* and *Ostrya carpinifolia*) and two oaks (*Quercus robur* and *Quercus pubescens*) leaves which are very common throughout European lowlands. We also compared the response of five freshwater amphipods and one isopod to leaf's characteristics and conditioning process. This study demonstrated that leaf toughness and the conditioning process are more important than geographical origin of leaves for determining shredder leaf litter consumption. The conditioning process tends to homogenize the consumption rate

of leaf litters and after several weeks of conditioning, the toughness of leave and their consumption rate did not differ. However, the sensitivity of shredders to the short conditioning period depend on the shredder species.

In a third experiment, we tested the hypothesis that harder leaves are not consumed early but could constitute a reservoir of trophic resources bioavailable later in the year. To do this we followed the leaf breakdown and leaf toughness evolution of three leaf species (alder, chestnuts and oak) with different conditioning duration in 8 streams (Western France). Our results confirm that soft leaves are quickly available for microorganisms and shredders, whereas hard leaves may constitute a reservoir of organic matter that is usable later, in the spring or summer. Our study suggests that a change in the diversity of riparian vegetation, in term of leaf toughness, induced by land use or climate change could shorten leaf litter availability across seasons. For example, in a climate change context, the replacement of soft leaves from trees living in temperate environments by harder leaves from trees adapted to more arid conditions could delay the availability of the feeding resource several months after abscission

and should be taken into account in riparian management.

In conclusion, the macroinvertebrate species identity, the litter type and the microbial conditioning level are important determinants in the litter consumption. Our studies highlighted that the leaf consumption are mainly macroinvertebrate species-dependent, whereas the transfer efficiency for other trophic levels is strongly controlled by the litter type.

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Diversity and distribution of freshwater amphipods from Tunisia

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ABSTRACT

Despite the vast surface of North Africa, the freshwater Gammaridae are still poorly known and only 21 species have been described until now (Rewicz et al., 2014). They belong to 3 genera: *Echinogammarus* (11 species), *Gammarus* (8 species), and *Chaetogammarus* (1 species). We can add to this list a brackish water genera that can be found in freshwaters close to the sea, *Rhipidogammarus* (1 species). The Tunisian freshwaters are known to harbor only 7 species: *Echinogammarus afer* Stock, 1974, *Echinogammarus dactylus* G. Karaman, 1987, *Echinogammarus simoni* (Chevreux, 1894), *Echinogammarus pungens* (Milne Edwards, 1840), *Echinogammarus tacapensis* (Chevreux & Gauthier, 1924), *Gammarus gauthieri* (S. Karaman, 1935) and *Rhipidogammarus rhipidiophorus* (Catta, 1878). To further document our knowledge of the freshwater amphipods from Tunisia, we sampled more than 60 sites (streams and springs) widely distributed in all regions of Tunisia. In this study, most of the previously reported species have been found except for *E. tacapensis* and *E. dactylus* which were not observed. However, our current surveys have allowed us to find at least 4 new species records for Tunisia, including at least three new species of *Echinogammarus*: and a new one for Tunisia: *Echinogammarus haraktis* Piscart, Merzoug et Hafid 2013, recently described in Algeria. Moreover, the sampling of these 9 species from the north to the south of Tunisia showed a heterogeneity in the distribution of species. Most of them are restricted to one region of Tunisia while only three species: *E. simoni*, *E. pungens* and *E. afer* were more widely recorded in rivers and water sources throughout Tunisia.

KEY WORDS

North Africa; geographical distribution; freshwater Gammaridae.

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Distribution of talitrid amphipods on the beaches of Crete: definition and role of different substrates and first report of *Deshayesorchestia deshayesii* (Audouin, 1826) (Talitridae) from Crete

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ABSTRACT

Occurrence of talitrid amphipods on the beaches of Crete was modeled, highlighting ecological categories which are complementary in terms of habitats.

KEY WORDS

Talitrids; island; sand-hoppers; beach-hoppers; *Deshayesorchestia deshayesii*.

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A sampling campaign was carried out during winter 2015, targeting presence and distribution of talitrid amphipods along the coastline of Crete, Greece. A total number of 31 beaches, selected to include a range of exposures and substrates were sampled for talitrid fauna, to be used as model taxa, consistent with literature (see Defeo & McLachlan, 2013 for a summary of current paradigms relating geomorphology and biotic patterns). Sampling in winter is a recommended practice for the establishment of baselines (Schlacher et al., 2008). The sampling method was trapping by pitfalls, suitable to be applied across different substrates and appropriate to estimate species' presence (Fanini & Lowry, 2016). Sampled talitrids were preserved in ethanol and identified using an interactive key (Intkey, Dallwitz, 2010) to world talitrids. A subsample was barcoded with Cytochrome Oxidase I (Hebert et al., 2003.). The species were then allocated into ecological categories, implying different relationships with the substrate they are found on (for Crete see Lowry & Fanini, 2013): sand-hoppers

substrate-modifiers, including *Talitrus saltator*) and beach-hoppers (non substrate modifiers, including *Deshayesorchestia deshayesii*, *Orchestia* sp.).

To classify substrates different than sand we applied the use of fractions, following Blott & Pye (2001) for categories of sand and pebbles found at the sampled localities. This allowed us to discriminate sand from mixed substrates, on pebbles beaches. The location of a beach on a northern or a southern coast was also considered as an informative variable, implying a different exposure to dominant winds (proceeding from NNW) and waves.

Presence/absence data of species identified were modelled with Generalized Linear Models (GLM), including a set of continuous (beach width; beach slope; substrate penetrability; substrate size: Mz), discrete (north/south exposure) and dummy (substrate type) variables. Model selection was based on Akaike Information Criterion (AIC). One model was obtained for the general category “talitrids” as a response variable, then two different models were developed for each of the two ecological categories.

Five species of talitrids were identified: *Talitrus saltator*; *Deshayesorchestia deshayesii* (first report of this species for Crete), *Orchestia gammarellus*; *O. montagui*; *O. stephenseni*. Information from barcoding fully supported morphology in the case of *Talitrus saltator*, while matches for the other species were less clear.

Independent variables were checked for correlation prior to modelling: width and slope were correlated, consistently to paradigms of sandy beach ecology (Defeo & McLachlan, 2013), and width was included in GLMs as a more informative variable. Results indicate that the presence of talitrids is significantly related to beach width with a positive mode. Other factors retained but not significant are substrate-related features: Mz and substrate type, with decreasing presence on coarser substrates and increasing presence when cobbles are present. Models highlighted differences between ecological categories, with the presence of sand-hoppers negatively related to coarser grain size. Their presence also increases on the southern coast, however not significantly. Beach-hoppers were instead found to be positively related to beach width, and decreasing on southern coasts. Beyond the first report of *D. deshayesii* and the modelling of talitrids presence on the coast of Crete, results allowed to discriminate substrate parameters related to the ecology of sand-hoppers and beach-hoppers.

While the model for talitrids points to a high plasticity of this group, present wherever there is habitat availability (as indicated by the positive relation to beach width), the two different ecological categories appear to be complementary in terms of

distribution and factors shaping presence/absence patterns.

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Ecophysiological performance of coexisting caprellids: new insights into mechanisms underlying invasive success

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ABSTRACT

Caprella scaura Templeton, 1836 (Caprellidae) is among the most widespread introduced species in the Mediterranean Sea where it seems to be displacing an ecologically similar congener, *Caprella equilibra* Say, 1818. In the context of a global changing environment, this study explores for the first time the physiological performance of the two species under different combinations of salinity and temperature to assess the importance of abiotic factors in modulating this interaction. Our results indicate that *C. scaura* is more eurytolerant than its congener at higher seawater temperatures. We overall demonstrate that physiology is playing a key role in the invasion success of *C. scaura* in this area.

KEY WORDS

Invasive species; physiology; stress tolerance; climate change; *Caprella scaura*.

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Biological invasions and climate change are considered two of the greatest threats for biodiversity conservation (Hellman et al., 2008). Furthermore, these global environmental changes may have interacting effects in the long term with climate-change conditions favoring the invasion success of introduced species (Sorte et al., 2010; Olabarria et al., 2016). However, few studies have examined the physiological mechanisms underlying this supposed differential success (Zerebecki & Sorte, 2011).

The skeleton shrimp *Caprella scaura* Templeton, 1836 (Caprellidae) is an introduced amphipod that has successfully spread along the Mediterranean Sea in a short time period where it seems to be displacing an ecologically similar congener, *Caprella equilibra* Say, 1818. A recent field-based

study highlighted the role of abiotic factors, especially salinity and temperature, in modulating this interaction and, therefore, in regulating the negative impact of this biological invasion (Ros et al., 2016). However, the effect of environmental stress on the physiological responses of these closely-related species is unknown.

During the present study, the differential species-specific stress tolerances to different combination of salinities (15, 25 and 35 psu) and temperatures (10°C and 25°C) in *C. scaura* and *C. equilibra* were compared with the aim to identify whether the invasive species is more stress tolerant than its ecologically similar congener and to predict the impacts of *C. scaura* under a future climate change scenario. Together with respirometric experiments and survival rate under different

temperature and salinity conditions, measures of hemolymph osmotic pressure were conducted. Additionally, reactive oxygen species (ROS) formation and antioxidant activities (catalase and superoxide dismutases) were also explored. Results indicate that the two species are osmoconformers. *C. scaura* is capable of tolerating exposure to 10°C with a much lower energetic expenditure than *C. equilibra*, which results in increased ROS formation for the later. Since this misbalance is not accompanied by changes in antioxidant defenses, *C. equilibra* suffers higher degrees of cellular damage and mortality rates under lower temperatures. At high seawater temperature (25°C) *C. scaura* also overpowers *C. equilibra*, tolerating a wider range of salinities. Moreover, while increasing temperature increases oxygen consumption rates in both species, at high temperatures the oxyregulatory capacity is solely maintained by *C. scaura*. All of this suggests that, compared to *C. equilibra*, *C. scaura* seems to cope better with both lower and higher temperatures. In the context of climate-changing conditions, an increase in water temperature may favor the invasion success of *C. scaura* in invaded habitats subjected to salinity fluctuations. Future studies addressing the complex interplay between environmental stress and physiological responses will improve our understanding of biological invasions in the context of climate change.

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Role of short-term colonization ability in the dispersal of fouling amphipods

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ABSTRACT

Dispersal is considered a key element of a species' invasiveness. However, few studies have addressed the influence of colonization ability in the dispersal of introduced species, especially in marine ecosystems. This study aims to explore if fouling amphipods with high potential for introduction (NIS, cryptogenic and exported species) show better colonization abilities than native species with a restricted distribution (and consequently low potential for introduction). Results from field experiments with artificial passive collectors deployed in the marina of Ceuta (north coast of Africa) in two periods in 2015 showed that amphipods with high potential for introduction exhibited higher ability to colonize the deployed structures in short time periods (<72 hours) than native species with restricted distribution. These findings suggest that colonization ability of artificial structures in amphipods can be related to their invasiveness.

KEY WORDS

Non-indigenous species; Ceuta; fouling; colonization ability; marina.

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Marine bioinvasions are considered as a key component of global change and are increasingly recognized as one of the greatest threats from human activity in the ocean (Carlton, 1996; Occhipinti-Ambrogi, 2007). Understanding colonization and spread of non-indigenous species (NIS) in marine ecosystems is therefore a priority for biological conservation. However, knowledge on the current status of marine NIS is still very fragmentary. Most studies are focused on sessile benthic invertebrates and seaweeds while smaller mobile organisms inhabiting these organisms are frequently overlooked (Chapman et al., 2005). Among the

small mobile epibionts associated with artificial submerged structures of harbors and marinas, focal points for the detection of NIS, amphipod crustaceans constitute one of the dominant taxa (Gavira O'Neill et al., 2016). Although likely vectors of introduction of exotic amphipods (such as hull fouling) are well known, transport of a species to a new geographical region is only the first step in the invasion process. Upon arrival, surviving introduced epibionts should dissociate from hull fouling communities on boats and migrate to recipient macrofouling communities to successfully colonize the new habitat. Few studies address this part of the

invasion process (Murray et al., 2011). Moreover, it is not clear whether colonization ability of small mobile epibionts is related to its invasiveness (i.e. inherent characteristics of NIS). Such information could be especially valuable in management strategies for early detection of epibiont NIS and controlling their spread. This study aimed to explore the contribution of marine amphipods to biological invasions in the North African coast of the Strait of Gibraltar, as well as to understand if their colonization abilities are related to their invasiveness. To address these aims, the abundances, composition and short-term colonization abilities of amphipods were studied during two periods, September 2015 (late summer) and January 2016 (winter), in the marina of Ceuta. For the first aim, scrapes of 20 x 20 cm of the fouling communities associated with the lateral surface of the floating pontoons were collected and for the second aim, standardized microhabitats made of commercially available plastic bath puff were deployed at one meter deep by a rope tied to the pontoons. These artificial collectors were retrieved after 3 and 15 days of immersion to explore short-term colonization abilities of fouling amphipods. A total of 27 amphipod species were found (23 using the artificial collectors and 17 in the scrapes). Seven species were classified as cryptogenic, due to their uncertainty related to their native range (Marchini et al., 2017), and two species were considered NIS in Ceuta: *Caprella scaura* Templeton, 1936 (found for the first time in Ceuta) and *Stenothoe georgiana* Bynum et Fox, 1977 (recorded for the first time in the northern coast of Africa). Species with high potential for introduction, i.e. widely distributed species associated with human transport vectors (NIS, cryptogenic and exported species) exhibited better colonization abilities of artificial collectors than native species with a restricted distribution (and consequently low potential for introduction). This may imply that a short-term colonization ability of artificial empty niches could be positively related to the potential for introduction of fouling amphipods and therefore with their invasiveness. This ability could facilitate a rapid secondary spread of the species by boats, even by those

that remain moored for short periods of time (<72 hours), facilitating the expansion of its distribution range. Therefore, this study suggests that colonization abilities of empty niches could be a major issue to explain the widespread of some human-mediated transported marine species.

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Barcoding of deep-sea Amphipoda from Kuril-Kamchatka area (NW Pacific)

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ABSTRACT

The results of molecular analysis of amphipods from Kuril-Kamchatka Trench and adjacent abyssal area.

KEY WORDS

DNA barcodes; abyssal; hadal; cryptic species.

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Amphipods are one of the most abundant abyssal invertebrate groups (after Polychaeta and Isopoda), however, still little is known about their diversity in the deep seas. There are only 400 benthic species of amphipods found below 2000 m worldwide of which 50 were recorded from abyssal of NW Pacific. Deep-sea invertebrates, especially crustaceans, are very fragile and can be easily destroyed already during sampling. Thus, obtaining proper morphological material is difficult. This makes DNA barcoding a precious tool for biodiversity assessment of deep-sea organisms. Moreover during species description it became very popular to include the species DNA barcodes. The aim of the present research is to study the molecular diversity of deep-sea amphipods from Kuril-Kamchatka Trench and adjacent abyssal plain. The material was collected using epibenthic sledge at

28 stations at the depth range 4830-9580 m. The identified amphipods belong to 22 families and were preliminary assigned to 65 morphospecies out of which almost half is potentially new to science. We have obtained over 500 DNA barcodes (COI sequences) that were automatically subjected to Barcode Index Numbers (BINs) in Barcode of Life Data System (BOLD). BINs cluster barcode sequences algorithmically into molecular taxonomical units that show high concordance to species. The analysis divided barcodes into 133 BINs of which 130 are new to BOLD. The BINs were represented mainly by one to a few sequences/individuals, only two of them were shared by more than 20 individuals. Up to 7 BINs were recognized within a morphospecies, indicating existence of potential cryptic species.

An unusual, new pardaliscid (Amphipoda Pardaliscidae) genus from abyssal waters of the NW Pacific, N Atlantic and SE Atlantic

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ABSTRACT The description of a new genus from the family Pardaliscidae distributed in scattered deep-sea localities.

KEY WORDS Pardaliscidae; deep sea; new genus; distribution.

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A very peculiar and distinctive new taxa of the amphipod family Pardaliscidae has been discovered from widely separated, disjunct deep-sea locations in the NW Pacific (Kurile-Kamchatka abyssal plain and Sea of Okhotsk), North Atlantic Ocean around Iceland and the SE Atlantic Ocean (Guinea Basin). The specimens were collected during various expeditions (KuramBio I, SokhoBio, IceAGE and DIVA-2) using epibenthic sleds. All of the specimens have been collected in deep water, varying from 2562 to 5780 m. This new genus is only the third pardaliscid to have an entire telson, the other two being *Parpano* J.L. Barnard, 1964 (Columbian Basin and Puerto Rico Trench) and *Eperopeus*

Mills, 1967 (NW Atlantic). The new taxon can be easily differentiated from both these genera as follows: in the remarkably strongly developed gnathopod 1–2 propodus, lined with 3–4 strong spines on the palm, deep coxal plates and the strongly broadened pereopod 6–7 basis. It is also a very small pardaliscid, ranging from 2–3 mm in body length. We tentatively give a brief description of the new taxa, include a distribution map of collection localities and include some figures, as it will be formally described elsewhere. An attempt to obtain DNA barcodes for studied amphipods was done, but barcoding succeeded only in the case of individuals from the Kuril-Kamchatka area.

Ampeliscid amphipods in the Canadian western Arctic

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ABSTRACT Physical and behavioural factors related to differing distributions of ampeliscid amphipods are examined on the Arctic Beaufort coast of Canada.

KEY WORDS Amphipods; Ampeliscids; Canada; Arctic; Beaufort.

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Ampeliscid amphipods can form dense beds in the Arctic, where they are important food resources for Gray Whales. Part of the Beaufort Sea shelf in the western Canadian Arctic supports a dense ampeliscid bed in the area of wind-driven upwelling. This study examines the composition and distribution of ampeliscid species across the shelf and slope. Eight species were found, which grouped into three coherent distributions at the 5% level (SIMPROF, Similarity Profiles analysis): (1) *Byblis* spp. - *Haploops laevis*; (2) *Ampelisca* sp. - *H. tubicola*; and (3) *Byblis gaimardii* - *H. sibirica*. At the 1% level, *Ampelisca macrocephala* was significant-

ly associated with group 1 and *Haploops* sp. with groups 2 and 3. The group 1 species were largely confined to the dense ampeliscid bed and were 2–5 x more frequent and 4–100 x more abundant than the other species. The other species occurred in the ampeliscid bed as well but in low abundance. A larger proportion of their abundance was elsewhere on the shelf and slope. Congeners lacked association, indicating that morphologically similar species had spatially distinct distributions, likely a function of differing environmental tolerances and resource use to minimize competition.

Time-Series observations of scavenging Amphipoda in the Abyss

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ABSTRACT

This study focusses on 22 baited-trap samples set on the Porcupine Abyssal Plain at 4850 m, between the years 1985 and 2016. We analyse changes in the scavenging amphipod community composition.

KEY WORDS

Amphipoda; Lysianassoidea; abyssal; scavengers.

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INTRODUCTION

Amphipods of the superfamily Lysianassoidea are numerically dominant and taxonomically diverse, and form one of the most important groups of necrophages in most deep-sea environments (Smith, 1985; Thurston, 1990; Wolff, 1971). Deep-sea necrophagous amphipods play a key role in the recycling and dispersal of concentrated nutrient inputs in the form of food-falls (Britton & Morton, 1994; Smith, 1985). The use of baited traps in the deep sea has a long history. Traps were deployed as early as 1888 from the *Hirondelle* during an expedition of the Prince of Monaco, and between 1892 and 1912, 21 deployments of baited traps deeper than 3000 m were undertaken (Richard, 1934). After these early studies, it was not until the advent of more advanced technology, that the use of baited cameras revealed an active guild of mobile scavengers comprised largely of crustaceans and fish. Despite numerous baited trap operations since that time, few studies have fully analysed replicate baited traps for species composition. In the North Atlantic Ocean, Thurston (1979) studied

amphipods from a single baited trap set at 3852 m and Thurston (1990) analysed 44 traps taken on three different abyssal plains and over a wide bathymetric range (3144–5940 m). De Broyer et al. (2004) analysed 31 baited traps from a wide bathymetric range (171–3739 m) in the Southern Ocean. Horton et al. (2013) analysed the scavenging amphipod faunal composition at a single depth (2500 m) in detail by fully analysing 12 samples taken over a 4-year time period. The current study focuses on the Porcupine Abyssal Plain (PAP) time series study site at 4850 m, which has been the focus of research by the Institute of Oceanographic Sciences and the National Oceanography Centre for the last 30 years (Lampitt et al., 2010). Inter-annual and seasonal changes in phytodetritus have been reported at the PAP (Bett et al., 2001; Lampitt et al., 2010) and there are ongoing studies of the influence of such changes on the deep-sea benthos (Billett et al., 2001; 2010). Here we provide an analysis of the changes in the scavenging amphipod community at an abyssal site since 1985, covering a period of well-characterised changes in the upper ocean.

MATERIAL AND METHODS

A total of 22 samples were collected using a variety of freefall baited amphipod traps set at the Porcupine Abyssal Plain site at 4850 m between the years 1985 and 2016. Most material was collected within two metres of the sea floor using a variety of free-fall devices. These have included deployments of a baited benthic camera system and purpose-designed amphipod traps. The trap-rigs generally consisted of a frame, traps, buoyancy, an acoustic release and a ballast weight. For samples collected since 2011, the rig included a benthic and an epibenthic trap. Bait consisted of two whole mackerel (*Scomber scombrus* Linnaeus, 1758) wrapped in muslin. Preserved amphipods were sorted, identified to the species level and counted in the laboratory. Diversity indices, including expected number of species for 500 and 1000 specimens, ES (500) and ES(1000), exponential Shannon, $\text{Exp}(H')$, and inverse Simpson's ($1/D$) were calculated on untransformed data and compared to mean annual volumetric flux at 3000m (as a measure of organic matter supply to the sea floor).

RESULTS AND CONCLUSIONS

Initial analyses of the scavenging fauna show that there are changes in the community composition through time. The dominant species has shown some variation over the time period studied. In 1985 and 1986, *Paralicella tenuipes* Chevreux, 1908 was dominant (comprising 31% and 49% of the sample). In 2012, 2013 and 2016 the dominant species was *Paralicella caperesca* Shulenberger et Barnard, 1976. In 1991 and 2011, the most abundant species was *Abyssorchomene chevreuxi* (Stebbing, 1906) (comprising 55% of the sample in 1991, and 68% and 57% of the two samples collected in 2011). *Eurythenes* spp. contributed a higher proportion of the samples in the years 1985, 1986 and 1997 making up 28%, 27% and 20% of the samples respectively, and subsequently was less abundant, with lowest numbers recorded in 2012 (2%) and 1991 (2%). A number of rarer taxa were also found although their contribution to the samples was small. These rarer taxa, including *Paracallisoma idioxenos* Horton et Thurston, 2015, *Haptocallisoma lemarete* Horton et Thurston, 2015, *Valettietta*

gracilis Lincoln et Thurston, 1983, and *Cyclocaris lowryi* Horton et Thurston, 2014, were found in significant numbers in 1997. Sample compositions in 1991 and 2011 are distinct from other years. In 2011, *Abyssorchomene abyssorum* (Stebbing, 1888) was represented in much greater abundance (24%) and numbers of *Paralicella caperesca* were much lower (7%). The two traps analysed in 2011 also had the highest overall abundances (43,238 and 32,793 individuals). Excluding these outliers, the average number of individuals caught in the traps was 2493 (± 2537 SD). Preliminary analyses of available data indicate a negative relationship between the exponential Shannon, inverse Simpson's and the mean annual volumetric flux at 3000 m (see Table 1), and we conclude that the scavenging amphipod community is less diverse when there is higher organic matter input.

Year of sampling	Flux ml m ⁻² d ⁻¹	ES(500)	ES(1000)	Exp(H')	1/D
2013	3.09	8.7	9.2	8.2	3.1
2013	3.09	6.5	7.0	4.7	2.4
2012	6.69	7.5	8.4	4.1	2.0
2012	6.69	5.9	6.7	2.1	1.3
2011	1.65	6.0	6.4	4.6	2.0
2011	1.65	5.6	6.1	5.3	2.5
1997	1.74	9.9	[10]	12.4	4.2
1997	1.74	9.2	9.9	8.9	3.3
1991	N/A	[7]	[7]	6.7	2.7
1986	N/A	6.8	[7]	6.1	2.9
1985	N/A	8.4	8.8	10.0	4.3

Table 1. Summary of calculated diversity indices (ES = Expected number of species for 500 and 1000 specimens, $\text{Exp}(H')$ = exponential Shannon, $1/D$ = inverse Simpson's), for year of sampling with mean annual volumetric flux at 3000 m in ml m⁻² d⁻¹.

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Measuring uncertainty of marine alien species: the case of marine alien amphipods worldwide

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ABSTRACT

The present work aims to address the uncertainty regarding marine alien species, by investigating the available knowledge on the taxon of amphipods (Crustacea Peracarida). The main objectives of this study are: i) to develop an inventory of marine alien amphipods worldwide; ii) to discriminate amphipod species with convincing valid alien status from species affected by uncertainty; and iii) to identify and assess the different types of uncertainty that are affecting marine alien amphipods.

KEY WORDS

Amphipod; alien species; cosmopolitanism; pseudo-indigenous species; cryptogenic species.

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INTRODUCTION

Biological invasions are currently considered one of the major threats to global marine ecosystems services and biodiversity (Occhipinti-Ambrogi, 2007). The growing scientific interest on this topic led to the development of several inventories in order to collect the large amount of information currently available (e.g. Olenin et al., 2014; Pagad et al., 2015; Pagad et al., 2016). These inventories represent an useful and effective instrument for science and conservation policy as long as they collect up-to-date and accurate information. However, several gaps of knowledge still characterise data on biological invasions: incomplete historical records, taxonomic issues, scattered biogeographical information and different levels of accuracy in the compilation of databases (Marchini et al., 2015).

Our aim is to quantify the uncertainty affecting alien species, focusing on crustacean amphipods, one of the dominant taxa in shallow-water marine ecosystems worldwide. The lack of pelagic larval

stages and the limited movement capabilities of both juvenile and adult amphipods indicate that the natural spread of a benthic amphipod between oceans can only apply to a few species associated with marine turtles. For this reason, amphipods represent a good model-taxon to investigate biological invasions.

MATERIAL AND METHODS

Our analysis focused on marine and brackish water amphipods (freshwater and terrestrial species have been excluded). To develop our list of marine alien amphipods a literature research was conducted, starting with amphipod species targeted as “aliens” in the literature. The survey has been performed using several combinations of keywords in Google Scholar. Regional inventories of marine alien species were also consulted. Thereafter, we concentrated on amphipod species not included in alien species inventories, yet occurring in disjunct

biogeographical areas. This task required the consultancy and cross-checking of regional faunistic inventories, taxonomic accounts, and handbooks from different world regions. More than 250 sources have been consulted, taking into account synonymies and misidentifications.

For each species with disjunct distribution, possibly related to human introduction, we assessed the global distribution in the main world ocean zones. Type locality and first record of a species in a region were never uncritically assumed as evidence of native origin. When enough information was available we divided species distributions in native and invaded range; for cryptogenic species (*sensu* Carlton, 1996) this distinction was not feasible.

Species were then classified into different categories. As regards their alien status: VS - Valid alien Status: species treated as “alien” in the literature with strong evidence of human mediated transport, DR - Doubtful Records: species treated as “alien” in the literature, yet presenting some degrees of uncertainty, PI - possible Pseudo-Indigenous species: pseudo-indigenous species (*sensu* Carlton, 2009) are alien species perceived as natives. As for the type of uncertainty affecting the assessment of the DR and PI cases, we distinguished between two categories: I - Identity (species affected by taxonomical issues) and A - Alien status (cryptogenic species and species where natural distribution appears more plausible than human-introduction).

RESULTS AND CONCLUSIONS

The total number of benthic marine amphipods included in the analysis was 105, belonging to 21 families. The majority of the valid alien species are in the families Corophiidae (10 species), Aoridae (9 species), Ischyroceridae (7 species), and Caprellidae (6 species). A total of 55 species (52%) provided evidence of valid alien status (VS) in at least one marine region; out of these, 27 species are clearly introduced to some regions, but their status is uncertain elsewhere (VS+DR, VS+PI, VS+DR+PI). The invasion histories of the species are heterogeneous, with many unique cases. Within

our analysis, 73% of species is affected by uncertainty at some level and would require further investigation. In details: 26 species have been classified as DR, 9 as PI and 15 species have been classified as both DR and PI; 29 species suffer taxonomic confusion (I), while 24 species have an uncertain alien status (A), and another 24 species are affected by both types of uncertainty. The highest numbers of valid records are distributed on the Pacific coast of North America, southwestern Pacific region, Atlantic and Mediterranean coasts of Europe.

The quantitative estimate of uncertainty based on amphipods as model taxon suggests that all the existing intricacies in taxonomy and species' distributions will realistically require intensive research efforts in order to be solved.

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Could ingested microplastics affect the concentrations of PBDEs in *Talitrus saltator* (Montagu, 1808) (Talitridae)?

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ABSTRACT

Microplastics (MPs) are considered emerging pollutants widespread in the marine environments. Marine organisms often ingest MPs by mistake and since plastic can sorb Persistent Organic Pollutant (POPs) from the environment, we hypothesized that ingested MPs could be able both to transfer chemicals to organisms and remove the pollutants already present in the body. Supralittoral amphipods, *Talitrus saltator* (Montagu, 1808) (Talitridae) were selected for laboratory experiments. Adult sandhoppers were fed with fish food mixed with polyethylene microspheres contaminated with a labelled polybrominated diphenyl ether (13C-labelled BDE-47). In a second test *T. saltator* was fed with food contaminated with 13C-labelled BDE-47 and successively with food mixed with MPs to evaluate if they can sorb the contaminant ingested. Preliminary results seem to indicate a potential double role of MPs both as source and sink of contaminants.

KEY WORDS

Microplastics; *Talitrus saltator*; PBDEs; pollutant transfer.

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The growing accumulation of microplastics (MPs) in the marine environment is an emerging issue both at regional and global scale. Polymers discharged in the environment are not pure substances, they are usually combined with additives to enhance their performance and to lower the production cost. From a chemical point of view, these substances are often classified as Persistent Organic Pollutants (POPs) because they persist in the environment and bioaccumulate through the food web. It has been proven that these pollutants can cause damages to endocrine systems, influencing the development, the mobility and the reproduction of various organisms (Jones et al., 1999; Kabir et al., 2015). POPs can be released during the plastic life cycle, from production to disposal, and they may

pose environmental and health hazard. Moreover, plastic debris are hydrophobic and, when they end up in sea waters, tend to sorb and accumulate POPs that are already present in the environment. Plastic debris, owing to their small size and presence in the marine ecosystem, are often mistaken for food and ingested by several marine animals. Here, we hypothesized that ingested MPs, could either transfer chemicals to organisms or clean them adsorbing pollutants taken from the diet. To test this hypothesis, the supralittoral amphipod *Talitrus saltator* (Montagu, 1808) (Talitridae), which occupies a key position in sandy beaches food chains, was selected for laboratory experiments. *Talitrus saltator* is known to accumulate POPs and swallow MPs in natural conditions (Ugolini & Ungherese, 2011),

peculiarity that makes *T. saltator* a good biomonitor particularly suitable for the project purposes. Adult individuals of *T. saltator* were sampled on the beach of the Natural Park of Migliarino, San Rossore, Massaciuccoli (Pisa, Italy). During the sampling campaign, sand samples were also collected, from the upper 3 cm of sand using a metal spoon, to assess MPs abundance in their habitat. Each sample was taken from an area of 1 m² and at regular intervals of 2 m. MP particles (detected particles > 60 µm) were found in each sample with a mean value of 330 ± 90 particle kg⁻¹ d.w. Most of the MPs detected in sand samples were fibers (range 58–79 %), followed by fragments (range 18–42%) and others (range 0–3%). Contamination tests were performed in the laboratory using polybrominated diphenyl ethers (PBDEs), a class of organobromine compounds having endocrine disrupting properties widely used as flame retardants in plastic materials. Sandhoppers were fed with dry fish food mixed with polyethylene microspheres contaminated with a labeled brominated diphenyl ether (BDE-47C13). BDE-47 is the main component of the commercial penta-BDE mixture, a flame retardant mixture banned in Europe (EU Directive 2002/95/EC) in 2004. The concentration of BDE-47C13 determined in MPs by chemical analyses (GC-NCI-MS) was 12693 ± 279 ng/g, a value similar to those found in plastic debris from coastal environments (Hirai et al., 2011; Browne et al., 2013). A labeled contaminant was chosen to perform the contamination experiments in order to eliminate potential background interferences and better control the bioaccumulation rate of the pollutant. In a second test sandhoppers were fed with food contaminated by BDE-47C13 and, later, with food mixed with clean MPs in order to evaluate if plastic can sorb the contaminant previously ingested by *T. saltator*. The first test showed

that sandhoppers are able to bioaccumulate ¹³C-labelled BDE-47 either from MPs and fish food. The ¹³C-labelled BDE-47 uptake found in *T. saltator* was close to 4%. This result seems to confirm the role of MPs as carrier of contaminants. The second test showed that the ¹³C-labelled BDE-47 mean concentration value found in *T. saltator* exposed to contaminated food and clean MPs was lower than that found in *T. saltator* exposed only to contaminated food. This second experiment supported the hypothesis that MPs ingested by *T. saltator* can sorb contaminants from the organism. Thus, these preliminary tests seem to indicate a potential double role of MPs both as source and sink of contaminants.

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Nutritional analysis of amphipods from marsh ponds of Southern Spain

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ABSTRACT

It is necessary to find new marine resources of good nutritional quality to be integrated in formulated diets for the increasing demands in aquaculture. This study demonstrates that amphipods of marsh ponds from Southern Spain have a good biochemical composition and are proposed to be cultured in IMTA systems.

KEY WORDS

Amino acid; aquaculture; fatty acid; lipid classes; major metal; trace metals.

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During the last decade, there is an increasing interest in the use of amphipods for aquaculture. Guerra-García et al. (2016) demonstrated experimentally that detritus (mainly composed of uneaten feed pellets and fish faeces released by cultured fish in fish farms and sea-cage structure) can be a nutritional adequate and cheap feed for caprellid amphipods, providing a source of both omega-3 and omega-6 fatty acids. The present study reveals another interesting example of potential IMTA systems combining the extensive culture of fishes and amphipods associated to the marsh ponds of Southern Spain (Fig. 1). The ponds are easily accessible from land, and algae, traps or other devices can be placed and replaced without great effort and with low costs. Marsh ponds are, consequently, promising sceneries to develop environmentally sustainable IMTA systems.

A nutritional study was carried out for main amphipods species - *Microdeutopus gryllotalpa*, *Monocorophium acherusicum*, *Gammarus insensibilis*, *Melita palmata* and *Cymadusa filosa*- at South Spain aquaculture terrestrial ponds. These species

showed a high protein content (up to 40%), high n-3 PUFA and phospholipid levels, and high levels of phosphatidylcholine, phosphatidylethanolamine and triacylglycerols (TAG), the latter significantly high for *M. acherusicum*. *Microdeutopus gryllotalpa* and *M. acherusicum* showed the highest proportion of lipids (19.15% and 18.35%, respectively). Isoleucine, glycine and alanine were the dominant amino acids in all species. In addition, amphipods collected from ponds showed low levels of heavy metals.

Therefore, amphipods of ponds are good candidates to be used as feed, and are proposed as a new sustainable economic resource to use in aquaculture. *Gammarus insensibilis* may be the best to be intensively cultured as an alternative feed resource because shows: 1) adequate PUFAS n-3 and PL composition; 2) high levels of glycine, alanine, tyrosine, isoleucine and lysine; 3) high natural densities; 4) large body size (≥ 1 cm) and 5) high concentration of Ca. Furthermore, a combined culture of amphipods and fishes in these marsh ponds seems a promising and environmentally sustainable

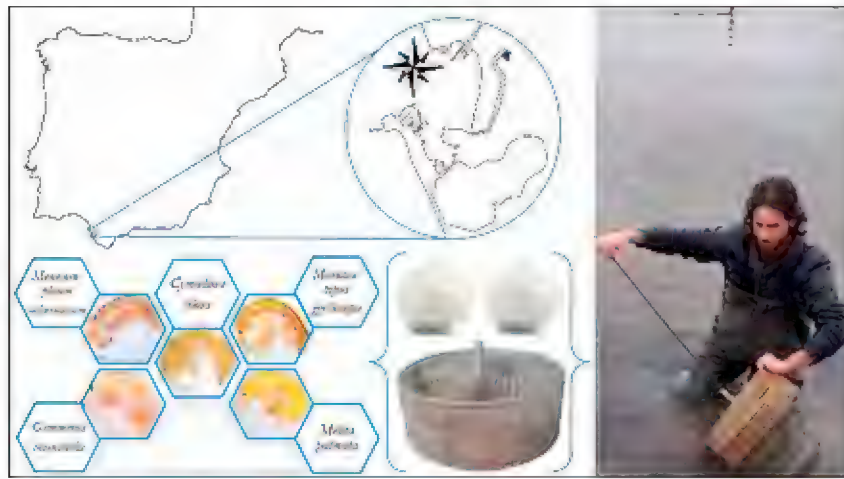


Figure 1. Location of the marsh ponds selected for the study in Southern Spain. Details of traps used to collect amphipods in the study area. Lateral view figures of amphipods species studied are taken from Arias & Drake (1999).

way to develop Integrate Multi-Trophic Aquaculture (IMTA) in these ecosystems.

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A molecular study on terrestrial talitrid amphipods (Crustacea) of the northeast Atlantic volcanic islands: new insights from the Azores

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ABSTRACT

An evolutionary scenario for the terrestrial talitrid lineages from the northeast Atlantic volcanic islands including the Azores, Madeira and Canary archipelagos.

KEY WORDS

Cryptorchestia; *Orchestia*; *Palmorchestia*; cytochrome oxidase I and II; histone H3.

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INTRODUCTION

The terrestrial talitrid amphipods from the Atlantic islands (Macaronesia) are of particular scientific interest. The species that are known to date from the Canary, Madeira and Azores archipelagos belong to the genera *Cryptorchestia* Lowry et Fanini, 2013, *Macarorchestia* Stock 1989, and *Palmorchestia* Stock et Martin, 1988. *Talitroides topitotum* (Burt, 1934) and *Talitroides alluaudi* (Chevreux, 1896) are two terrestrial species introduced accidentally by human activities.

The Azorean archipelago is composed of nine oceanic islands of volcanic origin, divided into western (Flores and Corvo), central (Graciosa, Terceira, Faial, São Jorge, and Pico) and eastern (São Miguel and Santa Maria, the oldest islands) groups. Currently two terrestrial talitrid species have been recorded from the Azores. According to the literature, *Macarorchestia martini* Stock 1989 is known only from the Gruta das Agulhas on the island of Terceira (Stock, 1989), while *Cryptorchestia chevreuxi* (De Guerne, 1887) was sampled from Faial (type locality) and the other major islands;

Cryptorchestia mateusi (Afonso, 1977) from Santa Maria was considered a junior synonym of *C. chevreuxi* (Stock & Boxshall, 1989).

Herein, we examine samples of terrestrial talitrid amphipods from the Azores collected by a research team directed by Paulo Borges (Universidade dos Açores, Terceira). We focus on phylogenetically unexplored specimens sampled in 2003 from Flores (west of the mid-atlantic ridge) and Graciosa (the northernmost of central Azores) because they showed clearly a small lobe on the posterior margin of the merus of male gnathopod 1, while *C. chevreuxi* from the islands of the Azores examined in previous studies (Stock & Boxshall, 1989) did not show this peculiar character (a criterion that indicated a taxonomic weight as compared to other structures, see below; Lowry & Fanini, 2013).

MATERIAL AND METHODS

We carried out a DNA sequencing of mitochondrial and nuclear protein-coding genes (cytochrome oxidase I and II, and histone H3, respectively) from

Cryptorchestia and other genera (including *Macarorchestia* and *Orchestia*) from the Mediterranean and Atlantic area (*T. topitotum* from Terceira as out-group species), as described in Davolos et al. (2017).

RESULTS AND CONCLUSIONS

Molecular data provided preliminary evidence that the talitrid specimens from Flores and Graciosa in the Azores belong to a distinct genetic lineage. This putative new taxon phylogenetically appeared to be distantly related to *C. chevreuxi* from Terceira examined in a recent study (Davalos et al., 2017). Previous findings indicated that the small lobe on the merus of male gnathopod 1 might have evolved independently on different events. In particular, among the Atlantic *Cryptorchestia* species (*C. canariensis*, *C. gomeri*, *C. guancha*, and *C. stocki* from the Canary Islands, *C. monticola* from Madeira, *C. chevreuxi* from the Azores) apparently only *C. stocki* (Gran Canaria) has a distinct lobe on the merus of male gnathopod 1. In contrast, the Mediterranean *Cryptorchestia* species as *C. ruffoi* (Rhodes Island), *C. cavimana* (Cyprus Island), *C. garbinii* (European regions), and *C. kosswigi* (Turkey) have clearly this small lobe (Davalos et al., 2017).

Notably, the putative new taxon occurring on the Azores was phylogenetically closest related to the two Canarian endemic species of *Palmorchestia* (*P. epigaea* and *P. hypogaea*) from La Palma, leading us to presume that they share an ancient common ancestor. *Palmorchestia* probably derived as a result of neotenic loss of the ancestral dimorphic gnathopods (see Villacorta et al., 2008, and references therein). Moreover, it is likely that the species *C. gomeri* (La Gomera), *C. guancha* (Tenerife), and *C. chevreuxi* with an apparent loss of the small lobe on male gnathopod 1 share a common ancestor with *Orchestia gammarellus* (from NE Atlantic and Mediterranean areas). The latter with *O. aestuarensis* (from Lanzarote, Canary Islands, and Kent, U.K.) and *O. mediterranea* (from NE Atlantic and Mediterranean areas) appeared to be more closely related to the Atlantic *Cryptorchestia* species than to the Mediterranean endemic *Orchestia montagui* and *Orchestia stephensi*, as reported in Davolos & De Matthaeis (2016).

Overall, it seems reasonable to assume that the

Atlantic volcanic archipelagos, with a sequence of island emergence and ageing over millions of years, have been colonized independently on different times, with some speciation events between and within the islands. This complex evolutionary scenario might shed light on the significance of the presence/absence of the small lobe on the merus of male gnathopod 1 in defining terrestrial talitrid lineages on the Canary, Madeira and Azores Islands, even within the same archipelago.

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When DNA barcoding fails: a case of intergeneric mtDNA introgression in freshwater Balkan amphipods

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ABSTRACT

DNA barcoding is a relatively new tool, which is very helpful in species delimitation, description and identification. However, the tool is far from being perfect. There are reports of mitochondrial genome introgressions as result of secondary contact between closely related species. Much rarer are, though, the mtDNA introgressions in case of species belonging to different genera, with significant morphological differences and long divergence history. Here we report such an introgression of mtDNA from *Laurogammarus scutarensis* Schäferna, 1922 (Gammaridae) a freshwater amphipod endemic to the ancient spring system of the Skadar Lake in the Balkan Peninsula, to *Echinogammarus thoni*, a species with much wider distribution around the Adriatic Sea encompassing both the fresh and brackish waters. The two species diverged ca. 7-9 Ma, in Miocene, while the introgression occurred very recently, not earlier than 0.5 Ma. Our findings put also in doubt the currently established taxonomy of *Echinogammarus* and validity of establishing *Laurogammarus* as a new genus.

KEY WORDS

DNA barcoding; freshwater amphipods; introgression; phylogeny; Balkan Peninsula.

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INTRODUCTION

DNA barcoding has proven to be an effective tool in providing molecular tags for animal species and even, although not without a debate, in species delimitation. This method involves the sequencing of particular loci (COI from mtDNA in animals) and identifying the species affiliation of the organisms based on these sequences. Despite its undoubted advantages, using it as the only alternative is pretty risky. There are cases that show that DNA barcoding method can fail. For example, the species having a

very recent common ancestor may still share mtDNA haplotypes. There are also reports of mitochondrial genome introgressions as a result of secondary contact between closely related species. Much rarer are, though, the mtDNA introgressions in case of species belonging to different genera, with significant morphological differences and long divergence history.

MATERIAL AND METHODS

A total of 147 specimens of *Laurogammarus*

and *Echinogammraus* were collected from 13 sites in and around the Skadar Lake basin (Montenegro and Albania) by researchers from the Department of Invertebrate Zoology and Hydrobiology (University of Lodz, Poland) during several scientific expeditions in autumn 2006 and spring/autumn 2014. DNA was isolated from muscle tissues by a standard proteinase K and phenol/chloroform extraction method.

For phylogeny reconstruction, a 538 bp long fragment of mtDNA COI gene from 147 specimens of *Laurogammarrus* and *Echinogammarrus* was used. Additionally, to verify the introgression hypothesis, a 698 bp long fragment of nuclear 28S rDNA marker was used. Then, amplified fragments were sequenced and used for a set of phylogenetic analyses such as (1) the analysis of nucleotide polymorphism (DnaSP), (2) reconstruction of NJ tree (MEGA), (3) reconstruction of time-calibrated phylogeny - chronogram (BEAST). The outgroup used was *Echinogammarrus veneris*, *E. pungens* and *E. berilloni*.

RESULTS

A total of 29 COI haplotypes were found in the dataset: 27 haplotypes were unique for *L. scutarenensis*, 1 haplotype was unique for *E. thoni* and 1 was shared between the two species. Phylogenetic analysis of the nuclear 28S rDNA revealed that the individuals of *E. thoni* bearing COI haplotype characteristic for *L. scutarenensis*, possessed the nuclear haplotype exclusive of *E. thoni*. That indicated a case of intergeneric introgression of mtDNA from *L. scutarenensis* to *E. thoni*. The time-calibrated reconstruction of phylogeny revealed that the introgression event occurred between lineages that diverged already ca. 17 My ago.

CONCLUSIONS

The assessments of biodiversity on the basis of DNA barcoding cannot be uncritically accepted. A wider phylogenetic context should be always taken into consideration.

Biological traits and potential vector of dispersion of the non-native amphipod *Gammarus roeselii* Gervais, 1835 (Gammaridae)

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ABSTRACT

This paper reports some experiments on biological traits of the non-native amphipod *Gammarus roeselii* Gervais, 1835 (Gammaridae), in the sub-lacustrine Ticino River basin (Lombardy, Northern Italy), assuming aquatic birds as potential vector of spreading. This species shows a very high resistance to desiccation in subaerial conditions and an ability to remain attached to duck legs even in presence of an air current.

KEY WORDS

Gammarids; non-native species; survival rate; aquatic birds; passive dispersal.

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INTRODUCTION

The amphipod *Gammarus roeselii* Gervais, 1835 (Gammaridae) naturally colonizes the freshwater bodies of the Balkan, Peloponnesian and Anatolian peninsulas.

Up to 2005, in Italy, its presence had been reported only for the Sile River basin (Lombardy, Northern-Eastern part of Italy); subsequently, in 2014, it was discovered in the Ticino River basin (Po River floodplain, Northern Italy), despite the lack of direct connection between these basins (Paganelli et al., 2015).

The presence of alien species in almost all freshwater ecosystems is often a consequence of the anthropogenic activities but other vectors could be responsible: aquatic birds are indicated as one of them. It is known that they can act as vectors of dispersion for plants but they also have an important role for the dispersion of the microbenthic fauna (Figuerola & Green, 2002).

Furthermore, some invasive freshwater species

have developed morphological and physiological adaptations to survive out of the water for a relatively long period of time and, therefore, this capability represents an advantage during the invasion process.

In order to verify if *G. roeselii* can survive in sub-aerial conditions and if aquatic birds may be vector of dispersion for this species, a series of laboratory experiments were performed.

MATERIAL AND METHODS

In order to test the resistance of *G. roeselii* in sub-aerial conditions (EXP-A), we performed two different experiments using a total of 50 specimens (length 10.26 ± 2.01 mm). For the first experiment (EXP-A1), 25 specimens were placed in Petri plates without water, at air temperature of 20 °C and 41% RH. For the second experiment (EXP-A2), we used the same number of specimens but we tested their resistance at 25 °C and 35% RH. For both experi-

ments, the time of death of each amphipod was recorded and a survivorship curve was created.

In order to test the role of aquatic birds as a vector of dispersion, we performed different experiments using a leg of *Chairina moschata* (Linnaeus, 1758), which have webbed paws that could better induce the potential adhesion of the amphipods (Rachalewski et al., 2013).

In the experiment EXP-B, we tested the amphipods potential adhesion to a duck paw immersed in the water for two different periods of time: a short (EXP-B1= 5 seconds) and long (EXP-B2= 20 seconds).

For both trial (EXP-B1 and EXP-B2), we performed 100 replicates using 25 specimens of *G. roeselii* for each of them. Then, at the end of each replicate's time, the duck paw was pulled out of the water and the clinging amphipods were counted.

Furthermore, in the experiment EXP-C we tested the ability of *G. roeselii* to remain attached to a duck paw out of the water. For the experiment EXP-C1, a total of 50 amphipods (10 specimens for each replicate) were placed on a duck paw and the time when every single specimen dropped from the paw was recorded; after 10 minutes, each replicate was considered finished.

Finally, in the experiment EXP-C2, following the previous experimental design, we tested the ability of *G. roeselii* to remain attached to a duck paw in presence of an air current at a speed of 12 Km/h.

RESULTS

The results of EXP-A1 and EXP-A2 show that all the specimens survived for at least 80 minutes out of the water, with a survival rate of about 90% for 120 minutes.

In the EXP-A1, the survival rate dropped at 50% after 190 minutes while in the EXP-A2 this value was recorded after 160 minutes, about 30 minutes earlier than the first experiment.

In the EXP-A1, 10% of the amphipods survived for more than 4 hours while, in the EXP-A2, one specimen survived for 6 hours out of the water. In both experiments, each amphipod died after they lost about 50% of the water of their body fresh weight.

In the EXP-B1 at least 1 specimen remained attached to the duck paw in 52% of the total repli-

cates, with a maximum number of 3 gammarids observed 3 times.

At the contrary in the EXP-B2 at least 1 specimen remained attached to the duck paw in 86% of the total replicates, with a maximum of 6 gammarids observed 2 times.

Finally, the EXP-C indicates that the success in the attachment of *G. roeselii* to a duck paw is influenced also by the presence of an air current. The EXP-C1 with no air current, shows that 50% of gammarids remain attached to the paw for 95 seconds and 14% of the total specimens were still attached after 10 minutes. At the contrary, the EXP-C2 indicates that 50% of gammarids remained attached to the duck paw for 110 seconds; moreover, after 10 minutes, 22% of the total specimens were still attached to the paw.

CONCLUSIONS

Our study provides experimental evidence for both the survival of *Gammarus roeselii* out of the water and the spreading of this alien species through aquatic birds.

We report that *G. roeselii* is able to survive out of the water for a long period of time with a survival rate that increases at lower temperature and higher relative humidity. This is possible thanks to the internal position of their gills, which can guarantee an adequate humidity for breathing.

Moreover, our data show that this species is able to remain attached to duck paws, even in presence of an air current. In our experiments the success in the attachment of *G. roeselii* is apparently higher in presence of an air current: this could be due to the presence of hooks on their legs and to their cling-behaviour when the paw is in motion.

All these physiological and behavioural characteristics seem to provide several advantages for this alien species during the colonisation process of new habitats, increasing its chance to enlarge its original area.

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Alien amphipod-driven transformations of macroinvertebrate assemblages in inland waters of the Baltic Sea basin

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ABSTRACT

Amphipod *Pontogammarus robustoides* (Sars, 1894) (Pontogammaridae) was found to tangibly affect macroinvertebrate assemblage composition and metrics in invaded Lithuanian lakes.

KEY WORDS

Pontogammarids; temperate lakes; community effects.

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In the 1960s, more than 100 Lithuanian lakes have been subjected to large-scale introductions of Ponto-Caspian peracaridan crustaceans in order to improve fish feeding conditions. Among these peracaridans, three amphipod species were introduced: *Pontogammarus robustoides*, *Obesogammarus crassus* and *Chaetogammarus warpachowskyi*.

The most successful among the species was the largest one, *P. robustoides* (Sars, 1894) (Pontogammaridae). Its currently known distribution among Lithuanian lakes appears to be mostly limited to large meso(eu)trophic lakes or lakes with high water turnover, probably due to decent oxygen conditions under winter ice cover. Previous studies have suggested that this amphipod is able to affect the diversity of littoral macroinvertebrate assemblages. As a result, applicability of national water quality assessment method, the multimetric Lithuanian Lake Macroinvertebrate Index (LLMI), may be limited in such lakes and in need of refinement. Using the nationally approved semi-quantitative method to collect macroinvertebrate samples from Lithuanian meso(eu)trophic lakes reflecting a gradient of *P. robustoides* abundance, we analysed its

effect on macroinvertebrate assemblage composition and the core metrics of the LLMI. Our data showed that on a mesohabitat scale, relative abundance of *P. robustoides* is of higher importance in shaping family-level composition of these assemblages with respect to so-called habitat-engineering mussel *Dreissena polymorpha*, which was present in all lakes. Proportions of predatory insect larvae without particular defence mechanisms seemed to be negatively related to *P. robustoides*, suggesting that pontogammarids may be winning the competition against such native insect predators and/or preying on them. Meanwhile, pontogammarids appeared to be associated with higher relative abundance of caddisfly larvae with stone-incrusted cases and some molluscs. Furthermore, the negative impact of pontogammarid relative abundance on estimated core macroinvertebrate metrics of the LLMI appeared to outweigh the positive impact of *D. polymorpha*. We propose that applicability of the national assessment method in large meso(eu)trophic lakes may be enhanced by excluding *P. robustoides* data from species lists before calculating the diversity index and the share of abundance

of sensitive taxa, and using family-level data to estimate the richness of sensitive taxa. Involving the

functional trait approach into the national method should be worth consideration in the future.

Molecular Confirmation of Invasive Leucothoid Amphipods

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ABSTRACT Molecular sequence data confirm the trans-Pacific distribution of *Leucothoe eltoni* Thomas, 2015 (Leucothoidae). This may increase our understanding of introduction paths utilized by invasive species.

KEY WORDS *Leucothoe*; amphipods; invasive species; molecular DNA; commensal.

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A species of amphipod crustacean originally described from the Indo-Pacific has been documented as an invasive species in Hawaii, U.S.A. Recent collections revealed morphologically identical specimens living on U.S. military piers in Okinawa, Japan. The objective of this research is to confirm

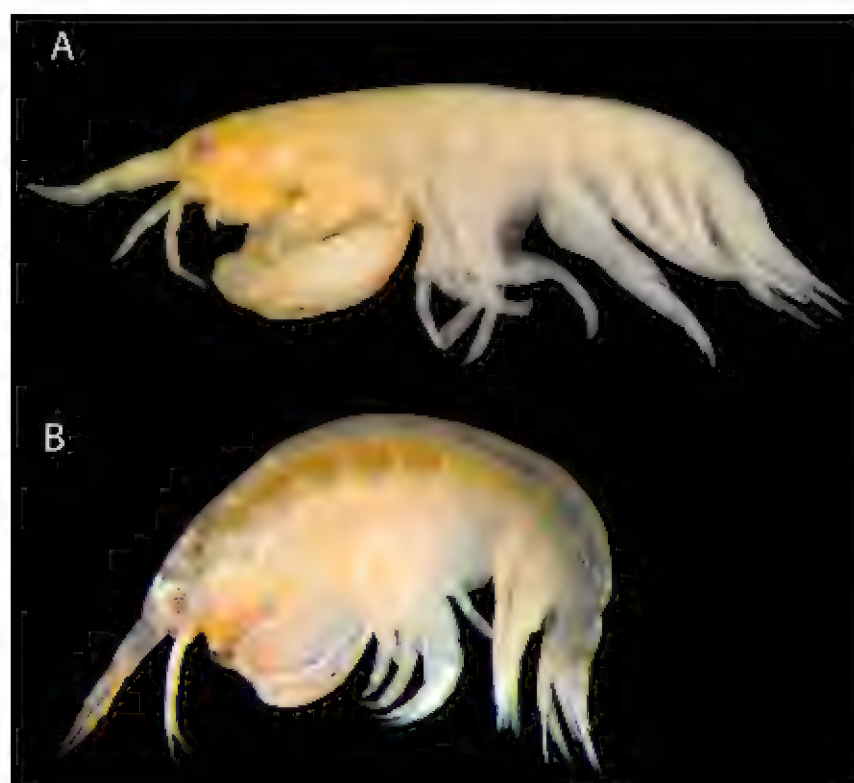


Figure 1. Male specimens of *Leucothoe* cf. *eltoni* collected from A. Maui, HI, U.S.A. and B. Okinawa, Japan.

the identity of morphologically identical amphipods collected from across the Pacific Ocean using molecular sequence data. Specimens of *Leucothoe* cf. *eltoni* Thomas, 2015 (Leucothoidae) (Fig. 1) were collected from Indonesia, Hawaii and Okinawa. DNA was extracted from 102 specimens (30 from Honolulu, HI, 30 from Maui, HI, 30 from Okinawa, Japan, and 12 from Indonesia). 18S rDNA and COI mtDNA were amplified using PCR and DNA was sequenced from 64 specimens (32 from Honolulu, HI, 21 from Maui, HI, 12 from Okinawa). Preliminary data suggest that the sequences are nearly identical between Hawaii and Okinawa specimens. Sequence data from the Indonesia specimens are in-

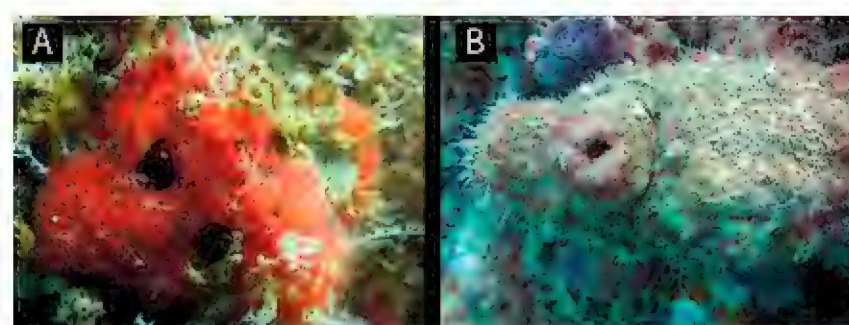


Figure 2. Examples of fouling hosts of *Leucothoe* cf. *eltoni*. A. *Monachora clathrata* sponge from Maui, HI, U.S.A. and B. *Pyura* sp. ascidian from Okinawa, Japan.

conclusive, and fresh material is required from the type locality to obtain valid DNA sequence data. However, the sequence data from the Hawaiian and Okinawan populations confirm that this species is invasive in Okinawa. Based on the establishment of

this species on piers and in harbors, the amphipods are most likely transported within their fouling hosts (Fig. 2) on U.S. military vessels. These results can be used to advance the understanding of the pattern of introduction that invasive species may utilize.

Hyperiid amphipods associated with siphonophores from the Gulf of Aqaba (Red Sea)

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ABSTRACT

In 5 species of Siphonophora collected in the northern Red Sea, 3 associated species of hyperiid amphipodes were found. Comments on their biogeography are presented.

KEY WORDS

Hyperiidea; Siphonophora; Red Sea; Gulf of Aqaba.

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Planktonic gelatinous cnidarians - Siphonophora - appear to be valuable target hosts for hyperiid Amphipoda. Long siphonophoran colonies offer plenty of living and hiding spaces. As very efficient predators, Siphonophora are granting amphipods with food in abundance.

In December 2013, in the northernmost part of the Red Sea - the Gulf of Aqaba, 90 colonies representing 11 species of Siphonophora were collected by snorkeling. In 5 of these species, cosmopolitan *Agalma elegans*, *Agalma okenii*, *Athorybia rosacea*, *Forskalia tholoides* and *Crystallomia* postlarvae 99 individuals of hyperiid amphipods were found. They represented three

species: *Parascelus edwardsi* (97 individuals), *Euthamneus rostratus* (1 ind.) and *Phronima colletti* (1 ind.). Amphipods were hiding in the nectophores or were buried deep in their mesoglea. Amphipod exuviae were also found in siphonophoran tissue. Nearly all these hyperiid individuals, inhabiting siphonophoran nectophores or bracts, were in a juvenile stage (2–3 mm in length).

Euthamneus rostratus and *Phronima colletti* are recorded for the first time from the Red Sea. Two siphonophorans - *Agalma elegans* and *Athorybia rosacea* appeared to be new host species for *Parascelus edwardsi*. *Phronima colletti* has been not yet recorded as an associate of Siphonophora.

Amphipod assemblages along shallow water natural pH gradients: data from artificial substrata (Island of Ischia, Italy)

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ABSTRACT Amphipods, studied along natural pH gradients, showed to be severely selected only below a tolerance threshold (i.e., pH 6.6), with important changes in assemblage structure.

KEY WORDS Amphipoda; ocean acidification; natural pH gradient; artificial substratum; Mediterranean Sea.

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Ocean acidification is one of the most serious threats facing marine life. It is likely to cause a decrease in biodiversity which may lead to shifts in ecosystem structure (e.g. Sunday et al., 2017). Amphipods, due to their structural and functional role, have become important in assessing biodiversity (Scipione, 2013a), and have long been known as sensitive environmental indicators (Conlan, 1994). The present study was framed within a wider research programme, aimed at investigating the effect of low pH/ $p\text{CO}_2$ on benthic assemblages, and in particular at evaluating amphipod robustness or sensitiveness to future acidification scenarios.

It was conducted at the Castello Aragonese, an area off the Island of Ischia (Gulf of Naples, Italy) characterized by natural pH gradients due to CO_2 volcanic vents. In this ‘natural laboratory’, amphipods were sampled by means of artificial collectors settled for one month at approx. 2 m depth. The present study is related to a comparison between collectors placed at two locations, in the water column and on the rocky reef, in May 2010 and June 2011, respectively. At both transects, three

stations along the pH gradients at the north and south sides of the Castello were established: St. 1 ambient (mean pH 8.1), St. 2 low pH (7.7; 7.8) and St. 3 extreme low pH (7.3; 6.9), totalling 42 samples (Ricevuto et al., 2014). Species richness, abundance and Shannon-Wiener diversity index were calculated for each sample. Data were analysed by means of nMDS and cluster analysis, and differences between location, side and pH were studied through SIMPER and PERMANOVA analyses.

Overall, 11,540 individuals (water column: 1,495; rocky reef: 10,045), belonging to 43 species (30; 37) were identified. Assemblage parameters showed significant differences between the two locations, with higher values on the rocky reef, except for St. S3. In the water column diversity increased significantly in the South at low and extreme low pH, while on the rocky reef it was significantly lower at St. S3 (Fig. 1a). The structure of assemblages: - showed clear disjunctions along the pH gradients in both the water column (ambient vs. acidified sites) and the rocky reef (ambient, low pH vs. extreme low pH) (Fig. 1b); - significantly differed between pH zones, with significant inter-

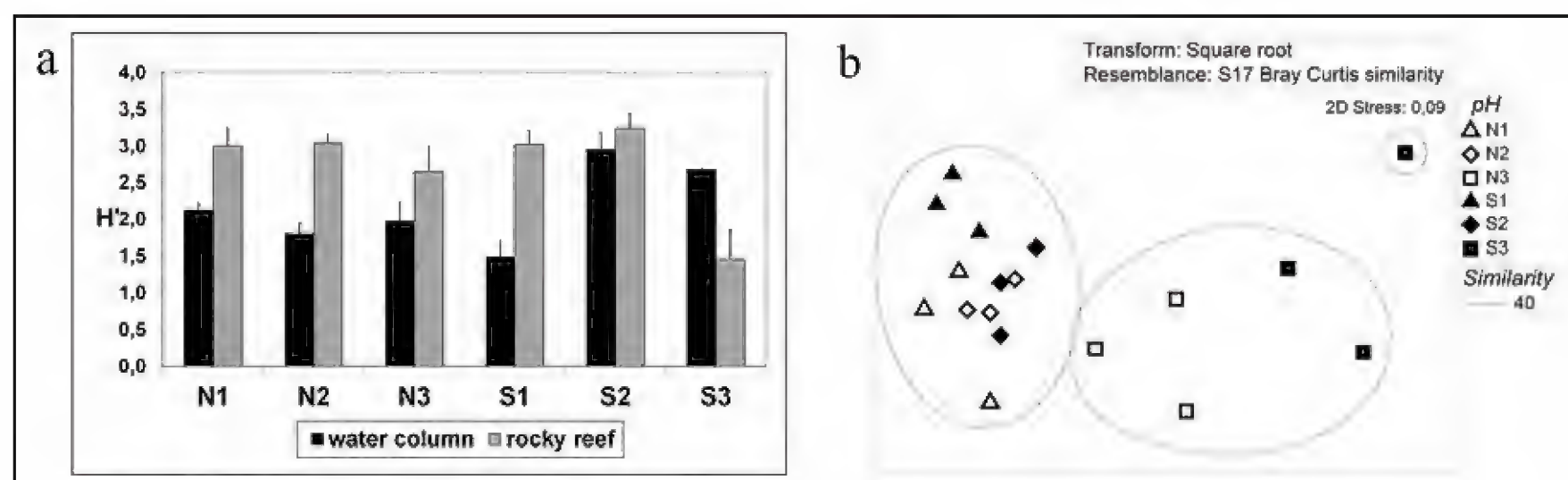


Figure 1. a. Mean values (\pm SE) of the Shannon-Wiener diversity index of amphipod assemblages in the two studied locations (water column and rocky reef), along the pH gradients (St. 1, 2, 3) at both sides (N: North; S: South) of the Castello Aragonese (Island of Ischia, Italy). b. Rocky reef: nMDS bi-dimensional plot produced from amphipod species-abundance data collected along the pH gradients.

actions (pH x side; pH x location) showing variations between North and South, and the two sampling locations. The taxa contributing mostly to dissimilarity are: - in the water column, the domicolous *Jassa* sp. (ambient), *Microdeutopus sporadhi* and *Ampithoe ramondi* (low, extreme low pH); - on the rocky reef, *Elasmopus pocillimanus*, *Apocorophium acutum*, *Protohyale schmidtii*, *Caprella acanthifera* (ambient, low pH), and *Gammarella fucicola* (66.8%, south side) and *Jassa oca*, exclusive at extreme low pH.

Amphipods showed patterns of colonization which varied according to location and pH conditions. In the water column, they are not affected negatively and appear to be tolerant to the low and extreme low pH values recorded, and differences may be due to the ecological requirements of individual species (Scipione, 2013b). Along the rocky reef, migrations from the adjacent vegetated substrata, as for the free-living *E. pocillimanus* and *P. schmidtii*, determine better structured assemblages; however, amphipods showed to be sensitive to the extreme low pH values (6.6) present at the south side. Therefore, assemblages are severely selected below a tolerance threshold, and only some species, such as *G. fucicola* (Zupo et al., 2015) and *J. oca*, seem to favor this extreme environment.

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A comparison of amphipod assemblages between canopy and understory strata in seaweed and seagrass beds off the coast of Otsuchi Bay, Japan

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ABSTRACT

This study compared the amphipod assemblages between the canopy and the understory of Sargassum, kelp and seagrass beds in Otsuchi Bay, Japan. Although the macrophyte biomass was larger in the canopy than in the understory in all the bed types, amphipods were more abundant and diverse in the understory in all cases, in particular, free-living amphipods. Tube-dwelling amphipods dominated both the canopy and the understory throughout the study period, except that algae-boring amphipods dominated the canopy of kelp beds in summer. The higher abundance and diversity of amphipods in the understory (even though there were lower habitat abundances than in the canopy) was probably due to the fact that the understory provided (1) more sheltered habitat for protection from predators and/or water movement, (2) more detrital deposition (a food source for amphipods) and (3) more diverse microhabitats.

KEY WORDS

Amphipod assemblage; canopy; seagrass bed; seaweed bed; understory.

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INTRODUCTION

Seaweed and/or seagrass beds provide one of the most productive ecosystems on earth (Costanza et al., 1997). Amphipods are one of the important components of these ecosystems, comprising a large number of species with high abundance and biomass. Furthermore, amphipods play a major role in transferring energy in seaweed and seagrass ecosystems as primary consumers and act as prey for higher consumers (Michel et al., 2015).

Seaweed and seagrass beds, even when dominated by a single macrophytic species, provide heterogeneous environments for amphipods; hence, they can be regarded as a complex of various habitat types, each providing specific microhabitats. Furthermore, these beds can be differentiated into

canopy and understory. Canopy and understory provide different biotic and abiotic environments, and as a result, their faunal assemblages also differ (Hirst, 2007); however, most previous studies have focused solely on amphipod assemblages in the canopy of macrophyte beds, and little attention has been paid to the amphipod assemblages occurring in the understory of those beds.

Therefore, this study aimed to characterise differences in assemblage structure and dynamics of amphipods communities between the canopy and understory of seaweed and seagrass beds.

MATERIAL AND METHODS

Sampling was carried out using SCUBA every

1–3 months, from July 2015 to June 2016, at Akahama in Otsuchi Bay, on the Pacific coast of north-eastern Japan. The following three types of macrophyte beds that occur on subtidal rocky shores were investigated: *Sargassum yezoense* (sargassum bed), *Saccharina japonica* var. *religiosa* (kelp bed) and *Phyllospadix iwatensis* (seagrass bed). Three quadrats (25 cm × 25 cm) were haphazardly set in each bed. Within each quadrat, seaweeds and seagrasses were cut with scissors at just above their holdfasts or rhizoids, and then were put into a 0.6 mm-mesh net along with associated benthic animals (These samples were defined as canopy samples). Moreover, the material left on the bare rock surface, such as holdfasts, rhizoids, undergrowth vegetation and sediments, were immediately suctioned using an air-lift sampler. The suctioned material and associated benthic animals were gathered in a 0.6 mm-mesh net. (These samples were defined as understory samples). All samples were then sieved through a 1 mm mesh, and then all the amphipods were collected and identified into the lowest possible taxon. All amphipod species were classified into the following four functional groups: tube-dwelling species, algae-boring species, free-living species and commensal species. The dry weight of each macrophyte species was determined. In addition, the abundance, species richness and Shannon-Wiener diversity index (H') of amphipods were calculated for each quadrat and compared between the canopy and understory strata.

RESULTS AND DISCUSSION

In all beds, macrophyte biomass was much greater in the canopy than in the understory stratum. Alternatively, mean abundance, species richness and H' of amphipods were higher in the understories. In canopies of sargassum and seagrass beds, the assemblages were mostly dominated by tube-dwelling species, such as *Jassa morinoi*, *Erichthonius pugnax* and *Sunamphithoe* spp., throughout the study period. In the canopies of the kelp beds, algae-boring species, *Najna consiliorum* and *Ceinina japonica*, were dominant during summer; however, they decreased in abundance as the kelps died-back during winter. In the understories of all the beds studied, tube-dwelling species were domi-

nant throughout the study period, although free-living species, such as *Quadrimaera pacifica* and *Pontogeneia* spp., were also abundant and sometimes dominated the assemblages. Commensal amphipods were rarely found in either the canopies or understories.

Although habitat abundance (as determined by macrophyte biomass) is lower in the understories than in canopies, the abundance and the diversity of amphipods were higher in the understories. This suggests that amphipod assemblages were strongly affected by the habitat characteristics. The higher amphipod abundances in understories may be due to the more protected environment (from predators) provided by understories and/or that water flows more slowly through the understory than through the canopy. Therefore, understories may provide better shelter for amphipods, especially for free-living species, which are thought to be more sensitive to predation and water-movement than other lifestyles such as tube-dwelling species. Moreover, more detritus, an important food source for amphipods, might be retained in understories, because less water moves through the understory. The higher amphipod diversity in understories was due to the higher abundance of free-living species found there. In addition, the understories provided more diverse microhabitats, in undergrowth vegetation and sediment, which probably promotes a more diverse array of amphipods.

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Pollution's effect on solar orientation behavior in *Deshayesorchestia deshayesii* Ruffo, 2004 (Talitridae) of the lagoon of Bizerte (North of Tunisia)

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ABSTRACT

Some samples of *Deshayesorchestia deshayesii* Ruffo, 2004 (Talitridae) population of Menzel Abderrahmane were treated with two pollutants: potassium bromate, sulfur, and were undergone some solar orientation experiments. Compared with a control sample, the results obtained showed a high perturbation in the circular distributions of the first samples.

KEY WORDS

Solar orientation; pollutants; *Deshayesorchestia deshayesii*; Tunisia.

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INTRODUCTION

Nowadays, beach pollution become more and more prominent, directly affecting several organisms, specially the talitrid amphipods. Since they are considered biological indicators of pollution, these organisms migrate in search of other stable environments (Jagminienė et al., 1991; Ungherese et al., 2012; El Gtari et al., 2012, 2014; Fialkowski et al., 2009; Scapini et al., 2002, 2015). However, few studies have been carried out to verify the effect of pollution on the solar orientation of the talitrid amphipod. The present study focuses on the effect of some pollutants on solar orientation behavior in *Deshayesorchestia deshayesii* Ruffo, 2004 (Talitridae), collected in Menzel Abderrahmane beach (Bizerte, North of Tunisia).

MATERIAL AND METHODS

In this approach, two pollutants, potassium bromate (KBrO₃) and sulphur (S), were used. The period of stay of the pollutant in the treated sand was 5 days for potassium bromate and 3 days for sulphur. These exposure lengths were selected due to the resistance of *D. deshayesii*. Solar orientation experiments were carried out on 10-16/5/2016 for potassium bromate, and 15/7/2016 plus 23/6/2017 for sulphur over three periods of the day: 9:00, 12:00 and 15:00. The experiments were carried out using a circular Plexiglas arena with 40 cm diameter and containing 72 traps. Each trap is separated from the other with an angle of 5°. The experiments were performed under two conditions: with the ability to see only the sun (S) or

simultaneously the sun and the surrounding landscape (L).

RESULTS

Analyzes of the orientation behavior of samples treated with potassium bromate and sulphur revealed a disturbance in circular distributions. Compared to a control sample tested on Menzel Abderrahmane's beach whose individuals were perfectly oriented towards the shoreline, the spiked treatments were in most cases oriented towards a direction close to the theoretical escape direction (TED). However, some have completely changed direction or diverted from the TED (V test). To know which factors and variables have affected the solar orientation behavior of *D. deshayesii*, the multifactorial Spherically Projected Linear Models (SPLM) were developed using angle as a response to the following factors and variables: pollutants, landscape view (with (L) or without (S)), temperature (20–28°C), humidity (40–97%), solar azimuth, zenith, atmospheric pressure (1113–1117 mbar). Backward selection of variables or factors was used to develop further models that were compared with the Akaike Information Criterion (AIC). The best model chosen is the one with the lowest AIC: Angle ~ pollutants (***) X (time of the day *** + azimuth *** + zenith*** + landscape) + humidity ***. Using this best model, the Likelihood Ratio Test (LRT) was applied to verify the importance of the factors influencing the observed orientation's variation. This test showed a very significant dependence ($p < 0.001$) of the orientation on the pollutant, in positive interaction with the time of the experiment, the solar azimuth, the solar zenith and finally the landscape's vision. The model also indicates that the variable that had the most significant effect after the pollutants was "humidity", but it acted alone, without interaction.

CONCLUSIONS

The current study shows that the used pollutants (potassium bromate and sulphur) influenced significantly the orientation behavior of *D. deshayesii*.

This influence was manifested by: a high dispersion level and, other times, with a total dispersion of studied samples, a significant deviation from the TED. From this study, we can also conclude that semi-terrestrial amphipods in particular *D. deshayesii* could be used as a biomonitor for beach pollution.

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A morphometric approach to the systematics of the arctic genus *Gammaracanthus* Spence Bate, 1862 (Amphipoda Gammaracanthidae)

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ABSTRACT

The diversity of *Gammaracanthus* Spence Bate, 1862 genus (Gammaracanthidae) in the Arctic is reassessed by using quantitative morphometric analysis and the variation of COI gene sequences.

KEY WORDS

Gammaracanthus; Arctic; phylogeography; COI; morphometrics.

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Gammaroid amphipods of the genus *Gammaracanthus* Spence Bate, 1862 are distributed primarily in circumpolar coastal waters and in some coastal lakes of the Arctic Ocean basin. The genus makes up the distinct family Gammaracanthidae Bousfield, 1989, which is thought to be a sister group to the hyperdiverse Holarctic Gammaridae sensu lato. The diversity of *Gammaracanthus* is comparatively quite modest, but how modest remains unclear. Two lineages (species) have invaded continental non-marine waters of boreal Eurasia during the past several million years, but different concepts of the basal marine diversity have been proposed. Suggestions of generic and subgenus-level subdivisions (Bousfield 1989) are contradicted by weak molecular differences and notions of one or two arctic species only, and by inconclusive diagnoses of taxa.

We are reassessing the diversity of *Gammaracanthus* by using quantitative morphometric analysis contrasted with variation of COI gene sequences, with an aim to dissect true systematic differences from possible environmental effects on

the various arctic populations whose habitats range from saline to freshwater. Traditional morphometrics of linear measurements is used to capture the diversity of the characters that were used in previous, traditional morphological diagnosis. At the same time, geometric morphometrics of selected body parts is used to characterize shape variation. Some 130 specimens from across the Eurasian Arctic seas were measured so far. The results of the approaches are somewhat contradicting. Geometric morphometrics recognizes clusters of populations that roughly correspond to the systematic structure described by Lomakina (1952), whereas linear measurements or molecular data do not always agree.

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Comparative phylogeography of *Gammarus roeselii* Gervais, 1835 and *Asellus aquaticus* Linnaeus, 1758 from the Ohrid, Prespa and Skadar lakes, Balkan Peninsula

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ABSTRACT

Comparative phylogeography based on molecular mitochondrial marker, for two freshwater crustacean species (amphipod *Gammarus roeselii* Gervais, 1835 and isopod *Asellus aquaticus* Linnaeus, 1758) from the Neogene Balkan lake system was investigated and established within a biogeographical context .

KEY WORDS

Ancient lake; Amphipoda; Isopoda; molecular phylogeny; mitochondrial marker.

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INTRODUCTION

The Balkan Peninsula represents a major world-wide biodiversity and endemism hotspot. It is also a region with the ancient European tectonic lacustrine systems such as Ohrid, Prespa and Skadar (Fig. 1), each with a history of more than one million years and its own set of endemic species. Interestingly, the ancient Lake Ohrid is almost directly connected via the Drim River to Lake Skadar, and to the Lake Prespa with subterranean karstic channels. Among the freshwater fauna, Peracarida crustaceans play an important role in benthic invertebrate communities of lacustrine systems. They are also known for their high level of cryptic diversity (Sworobowicz et al. 2015; Grabowski et al., 2017).

The aim of our study is to reveal and compare the molecular population structures of two Peracarida species widely distributed in Europe, the amphipod, *Gammarus roeselii* Gervais, 1835 and the isopod, *Asellus aquaticus* Linnaeus, 1758 (Karaman & Pinkster, 1977; Sket, 1994), inhabiting the

three aforementioned ancient lacustrine systems, as well as to compare their phylogenetic relationships.

MATERIAL AND METHODS

A 530 bp long portion of the mitochondrial cytochrome oxidase I (COI) marker from specimens collected from 28 locations from Ohrid, Prespa and Skadar lakes were amplified. The reconstruction of phylogeny was inferred using Bayesian inference (BI) and Maximum Likelihood (ML) method in BEAST 1.8 and MEGA 6, respectively. Relationships between haplotypes in populations from the presumably newly colonized part of Europe were illustrated with Median Joining Network (MJ) constructed in NETWORK v5.0.0.1.

RESULTS

We observed that, in case of both analysed spe-

cies, the drainage systems of all three lakes are inhabited by endemic lineages, possibly cryptic species, that are not present elsewhere in Europe and are also spatially limited on the scale of the Balkan Peninsula. Interestingly, on the local scale the molecular diversity pattern is different in each species. In the case of *G. roeselii*, all three lakes are inhabited by the same lineage with only weak signal of differentiation between the basins. It witnesses high dispersal potential of this species, via the river con-

necting Skadar and Drim or by ectozoochory between the Ohrid and Prespa. In *A. aquaticus* we observed that the basins were inhabited by separate, endemic lineages. The above results will be discussed within a wider biogeographical context.

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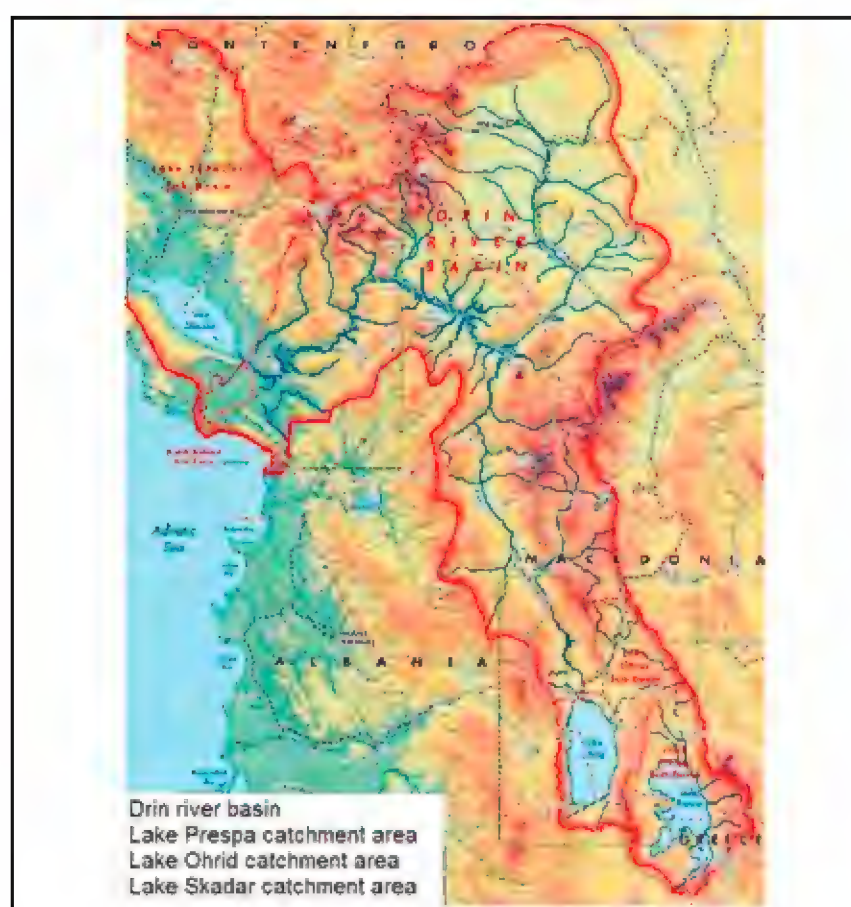


Figure 1. Map of the Lake Prespa, Lake Ohrid and Lake Skadar and its catchment area (Talevsky et al. 2014).

From There to Here: Tales and Travels of Two Invasive Leucothoid Amphipods

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ABSTRACT

Noteworthy occurrences of two invasive species of Leucothoid amphipods in sponges and ascidians from Raja Ampat and New Zealand marine environments

KEY WORDS

Leucothoe; commensal; amphipods; sponges; tunicates; invasive species.

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Invasive species are an increasing concern in marine biodiversity studies in the face of potential climate change scenarios. Sessile invertebrates such as sponges and tunicates are common marine invasive species that frequently house endosymbionts including leucothoid amphipods. The author recently described *Leucothoe eltoni*, Thomas, 2015 from *Herdmania* tunicates in Raja Ampat, Indonesia, and also recorded it from *Polycarpa* sp. tunicates in the Philippines. With a distinct shoe-shaped first gnathopod *L. eltoni* is reported as non-indigenous in Hawaiian waters found in the sponges *Mycale grandis* and *Monanchora clathrata*. Its introduction to Hawaii can be traced to a US Navy drydock transported to Pearl Harbor from the Subic Bay Navy base in the mid-1990's. Subsequently it spreads to several outlying islands. Investigations of *Leucothoe nagatai*, originally described from Japanese waters in the tunicate *Styela plicata*, show it to be invasive in California and New Zealand waters. In California the tunicate *Ciona robusta* is

the preferred host while in New Zealand *L. nagatai* is only reported from the tunicate *Styela plicata* and appears confined to harbors. *Leucothoe nagatai* exhibits a distinctive color pattern in freshly collected material, a stubby dactyl on the first gnathopod, and shortened antennae. Molecular evidence, 18S and COI, confirms that Japanese, California, and New Zealand species are identical. In both amphipod species transport by U.S. Navel vessels and merchant shipping are implicated. Issues of cryptic diversity of both invertebrate hosts and their commensal amphipods have hindered taxonomic clarification of these relationships and potential impacts in marine ecosystems. Sampling of diver-collected, freshly examined material is encouraged. Increased attention to leucothoid amphipods and their host distribution and ecology are certain to reveal additional details. Establishing sampling and reporting networks are discussed and updates on recent research will be presented.

Amphipods in transitional waters of Lithuania: roles and trends

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ABSTRACT

Amphipod assemblages in transitional waters are very dynamic and usually shaped by biological invasions. This is clearly seen in Lithuanian transitional waters located in the south eastern Baltic Sea basin. First alien amphipods appeared in the 18th century, and for the last 50 years amphipod assemblage is actually exclusively represented by alien species with the last invader recorded in 2015. Amphipods mostly are omnivores with different propensity to carnivory which seems to depend upon species identity. The last invader, the Killer Shrimp, is expected to dramatically reshape amphipod assemblages in transitional waters of Lithuania.

KEY WORDS

Curonian Lagoon; pontogammarid; *Dikerogammarus villosus*; assemblage composition; diet.

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Transitional waters due to their often location close to the marine ports contain the most dynamic crustacean assemblages. Alterations usually are induced by biological invasions frequently associated with marine shipping. The purpose of this work was to retrospectively review amphipod assemblages in Lithuanian transitional waters and investigate therein the trophic roles of main species of these crustaceans.

Transitional waters of Lithuania located in the south eastern Baltic Sea basin include the Curonian Lagoon and the mouth of the Šventoji River (Fig. 1). The Curonian Lagoon is a large (area 1584 km²), shallow (mean and max. depths are 3.8 and 5.8 m), largely fresh water but with salinity varying from 0 to 7 ppt in the northern part, eutrophic water body. The Šventoji River is a medium-sized river emptying directly into the sea. Data on amphipod assemblages were obtained by different conventional sampling methods during purposeful and occasional studies performed in the last 20 years. Information

on trophic roles of amphipods in studied ecosystems is based on stable isotope analysis. For this work, we used original results and already published information.

First alien amphipods reached Lithuanian transitional waters at the end of the 18th century when the basins of the Nemunas (Baltic Sea drainage) and Dnieper (Black Sea drainage) rivers were connected by the Oginsky Canal. This historical invasions brought the three species: *Chelicorophium curvispinum*, *Chaetogammarus ischnus* and *Gammarus varsoviensis*. It should be noted that *G. varsoviensis* only recently has been hypothesised to be a cryptic historical invader of Ponto-Caspian origin, and the amphipod *C. ischnus* recently has not been seen in Lithuanian waters (Arbačiauskas et al., 2011; 2017).

The second invasion wave consisted of deliberately introduced three species, two pontogammarids *Pontogammarus robustoides* and *Obesogammarus crassus*, and the gammarid *Chaetogammarus*

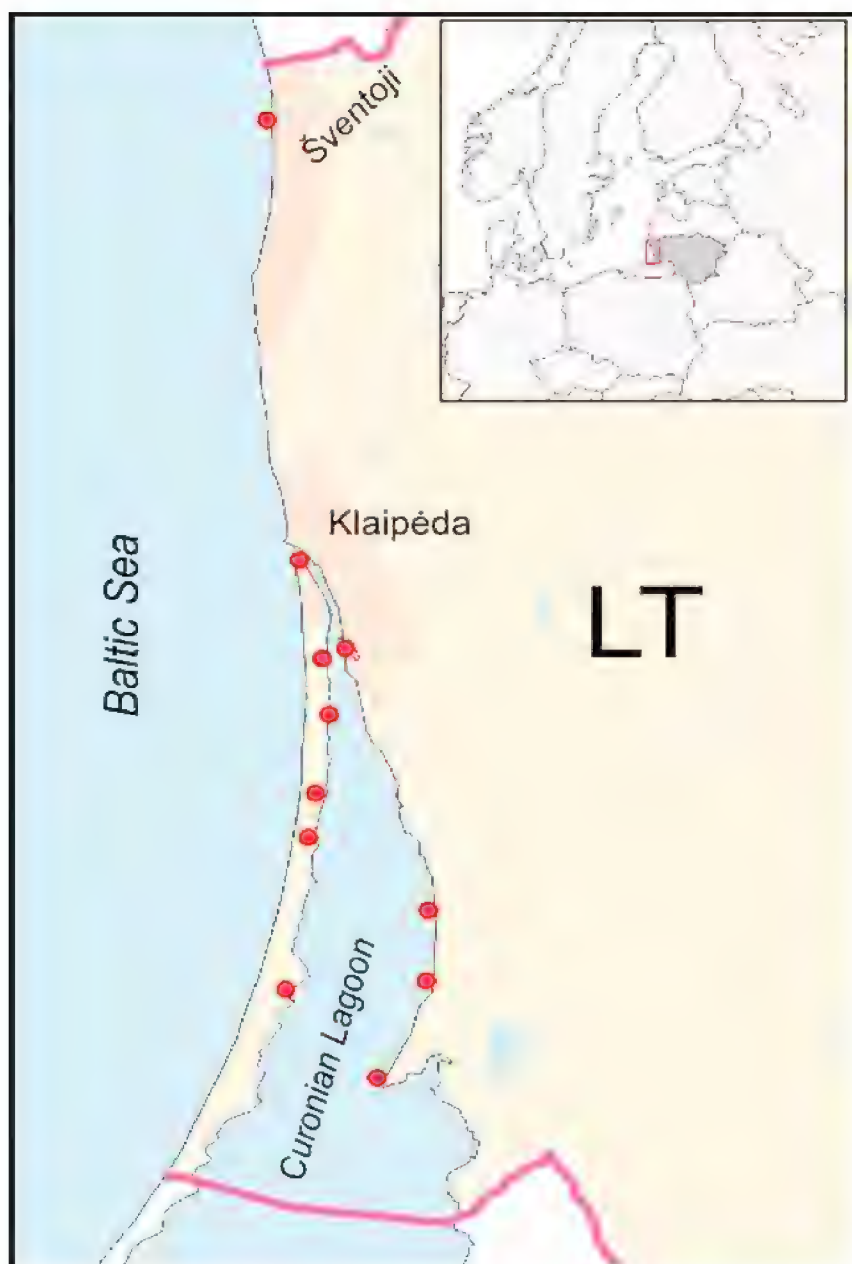


Figure 1. Localities of the new invader the Killer Shrimp in Lithuanian transitional waters in 2016.

warpachowskyi. These amphipods were transferred from Ukrainian waters to the reservoir located on the Nemunas River, and reached the lagoon by downstream dispersal in 1962 (Arbačiauskas et al., 2011).

Modern invaders are so far represented by two species, the North American gammarid *Gammarus tigrinus*, which was first recorded in 2004 (Daunys & Zettler, 2006), and the Killer Shrimp *Dikerogammarus villosus* first observed in the Curonian Lagoon and the mouth of the Šventoji River in 2015 (Šidagytė et al., 2017). In 2016, this new invader was found to be dispersed throughout the Curonian Lagoon (Fig. 1).

Stable isotope analysis showed the dominant amphipod species to be omnivores with propensity to predatory feeding increasing with body size. Stronger carnivory was seen in large-bodied species *P. robustoides* and *D. villosus*.

Recently, the amphipod assemblage was dominated by *P. robustoides* in the freshwater part of the Curonian Lagoon and *O. crassus* in the northern part of the lagoon, and co-domination of *G. tigrinus* and *P. robustoides* was a characteristic of the mouth of the Šventoji River. Invasion of the highly ecologically aggressive Killer Shrimp suggests that substantial transformation of amphipod assemblages of transitional waters can be expected in the nearest future.

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Gammarids parasites in small lakes of western Siberia

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ABSTRACT

Parasites of *Gammarus lacustris* G.O. Sars, 1864 (Gammaridae) were investigated in 23 lakes of the south of West Siberia. In populations are infected 28.7% individuals with *Hymenolepis nana* (12.3% of infected individuals), *Tetrameres fissispina* (15.3%), *Streptocara crassicauda* (31.6%), *Polymorphus magnus* (28.2%) and *Plagiorchis laricola* (12.6%).

KEY WORDS

Amphipoda; *Gammarus lacustris*; parasites.

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INTRODUCTION

Gammarids are sources of animal protein in bird nutrition. Between the birds and the gammarids there are stable trophic relationships. The distribution of gammarids in lakes along bird migration routes promotes the spread of invasive parasitic diseases. Gammarids, along with molluscs, are mostly infected with helminthes.

Gammarus lacustris G.O. Sars, 1864 (Gammaridae) is an intermediate and reservoir host for a significant number of wild birds' parasites in the southern part of the West Siberian Plain. This is due to the omnivorous nature of gammarids feeding in the studied lakes. The objective of the study was to determine the species of parasites infecting populations of *G.lacustris*. This is a first step for further study upon the epizootic situation in the lakes of the region. The study is devoted to parasite fauna of gammarids and distribution dynamics of parasites dangerous for birds and humans.

MATERIAL AND METHODS

The collection of gammarids was carried out using standard hydrobiological methods for sampling benthos and plankton. In the study of *G. lacustris* individuals in laboratory using digital microscopy, the presence of different stages of parasites development was determined inside the gammarids organism. The study was carried out by the method of parasitological dissection. Infection with helminthes was assessed by morphometric features and prevalence (proportion of infected individuals as a percentage of the total number of studied individuals).

RESULTS AND DISCUSSION

The main habitat for *Gammarus lacustris* in the Ishim plain are lakes overgrown with reeds, small in size (0.5–2.5 km²), shallow (average depth of 1.5–2.5 m) and brackish (6–20 ‰). There are favourable conditions for food and nesting of 44 migratory

birds species (*Podiceps nigricollis*, *Anser anser*, *Anas platyrhynchos*, *Anas strepera*, *Aythya ferina*, *Aythya fuligula* at all). Distribution of *G. lacustris* across the Ishim plain is positively correlated with the migration routes of birds.

The study of the population structure of gammarids was carried out during 2015–2016 in the lakes of the forest-steppe zone of Western Siberia for calculation of the limits of their fishing. Altogether 3278 individuals of *G. lacustris* were examined. The total infection rate was 28.7% of individuals with parasites such as the cestodes *Hymenolepis nana* and *Tetrameres fissispina*, the nematode *Streptocara crassicauda*, the acanthocephalan *Polymorphus magnus* and the trematode *Plagiorchis laricola*.

Migratory birds are usually definitive hosts, in the body of which parasitic worms reach sexual maturity and reproduce sexually. Gammarids can be both secondary intermediate hosts (the immature stages of the parasite develop in the body) and reservoir hosts (helminthes larvae do not develop, retaining invasive properties) (Churina, 1964).

Gammarus lacustris is the reservoir host for *Hymenolepis nana* (12.3% of the examined infected individuals). Birds become infected during spring migrations by eating gammarids. Cestodes injure the mucous membrane of the birds' intestines, violate its motor and secretory activities. Infected meat of wild birds, where an adult parasites develop, can be consumed by people.

The gammarids become infected with a nematode *Streptocara crassicauda*, when individuals of *G. lacustris* consume the eggs of this parasite. Larvae develop inside the body of *G. lacustris* (31.6% of the infected individuals studied). The larvae acquire invasive properties after two molts during two weeks when they reach the stage of a cyst (size 3.0–3.3 mm) localized in the dorsal part of the amphipod. Infected individuals are eaten by waterfowl (the definitive host) or by fishes (reservoir host).

Tetramerose (damage of the birds' gastric mucosa with a cestode *Tetrameres fissispina*) develops in the absence of flow in lakes with a high population density of ducks during migrations. The parasite affects mainly young birds. Eggs of the parasite enter the body of *G. lacustris* (15.3% of the examined infected individuals) together with fecal pellets. The development of the larva (as with streptocarose) occurs for two to three weeks at a temperature of 13–16°C. The larva becomes invasive after

two molts. Larvae remain viable in the body of gammarids during the life cycle (12–13 months). Infected individuals are eaten by waterfowl (the definitive host) or by fishes (reservoir host).

Another disease of wild ducks (the definitive host) is polymorphosis. It occurs when the birds are infected with *Polymorphus magnus*. The parasite is localized in the small intestine (Amin, 1985). The intermediate host is *G. lacustris* (28.2% of the all infected individuals). Eggs of parasite enter the body of crustaceans with fecal pellets. They pass through the stage of the acanthor in the intestine of gammarids, the stage of the preacanthella develops in the body cavity and becomes an invasive larva (acanthella). The process lasts 1.5–2 months. Infection of birds occurs when they eat gammarids.

The development of metacercariae trematodes *Plagiorchis laricola* occurs in the body *G. lacustris*. This species of amphipods is an additional host (12.6% of the investigated infected individuals). Gastropods are usual intermediate hosts are. The definitive hosts in the lake ecosystems can be birds and amphibians (Skryabin & Antipin, 1958).

When studying the life cycle and ecology of the causative agent *Opisthorchis felinus* opisthorchiasis it was noted that *G. lacustris* reduces the risk of opisthorchiasis (Strugova, 1994). It can swallow up to 90 eggs and 4 cercariae of the parasite per day (up to 10% of its dietary spectrum). In lakes of the forest-steppe zone of the south-west part of Western Siberia, it is noted that gammarids are the best eliminators of *Opisthorchis felinus* among crustaceans, causing the death of 83% of eggs after passing through the digestive tract of amphipods.

CONCLUSIONS

Samples from 23 lakes of the forest-steppe zone of the West Siberian plain south-west part have been studied. The parasitofauna of gammarids is represented by five species of parasites (*Hymenolepis nana*, *Tetrameres fissispina*, *Streptocara crassicauda*, *Polymorphus magnus*, *Plagiorchis laricola*). An initial assessment of the occurrence of these species at different stages of their life cycle is provided. The work will continue in the future and will focus on biological safety of commercial products of gammarids and epizootic significance of parasites for the region.

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Faunistic complexes of Lakes in Western Siberia forest-steppe zone with *Gammarus lacustris* G.O. Sars, 1864 (Gammaridae) domination

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ABSTRACT

Gammarus lacustris G.O. Sars, 1864 (Gammaridae) is a species-edificator of zoocenosis of lakes. Gammarids are regulators of the population of plankton crustaceans and biodiversity of the the small lakes ecosystems of the West Siberian Plain forest-steppe zone.

KEY WORDS

Gammarus lacustris; faunistic complexes; biodiversity.

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INTRODUCTION

The biological diversity of small lakes invertebrate communities was studied in the south-western part of the West Siberian plain. They form a specific system of trophic and topical interactions with the dominance of gammarids. The objective is to study the various biotic components of such lacustrine ecosystems and to describe their faunistic complexes.

MATERIAL AND METHODS

We used an integrated approach that allows us to fully assess the state of different types of ecosystems when studying the qualitative and quantitative characteristics of hydrobionts (Schwoerbel, 1970; Wetzel & Likens, 2000). The primary intracellular and extracellular products were determined during the growing season on different lakes using the radiocarbon method (Sorokin, 1999). The secondary production of zooplankton and zoobenthos as well

as bacterioplankton and its production was assessed according to the standard methods (Yezdani, 1985; Harris et al., 2000; Kemp et al., 1993). To calculate the secondary production, we used the P/B-coefficients obtained experimentally for the populations of organisms living in the lakes of the region. The taxonomy of the studied invertebrate hydrobionts is given in accordance with the identification keys (Borutsky et al., 1991; Belyakova, 2006; Makarova & Gogorev, 2006; Alekseev, 2010; Kutikova et al., 2010; Bledzki & Rybak, 2016).

RESULTS AND DISCUSSION

In the forest-steppe zone of the Kurgan region (south-western part of the West Siberian Plain) there are 4202 lakes of different genesis, each with an area of more than 0.01 km². The total area of the lakes is 2770 km² (Electronic catalogue, 2012). The lakes make up 3.8% of the region total area. These are lakes (87.3% of the total number) with an unstable hydrological regime, often drying out or in

the course of succession, turning into bogs. The 88.5% of the lakes are slightly mineralized (up to 1.0 ‰), 9% - brackish (1.0–10.0 ‰), 2.5% - bitter-saline (more than 10.0 ‰). A 47% of the lakes (from the total lakes area) are located to the west of the Tobol River in the Tobol-Iset interfluvium, 53% - in the Tobol-Ishim interfluvium. The region's fisheries fund includes 1473 lakes with a total area of 1386 km², of which 60% are used for industrial fishing and fish farming.

Among the lakes, there are small (1.0–1.5 km²) and deep (up to 3–4 m) lowland reservoirs with shallow banks, overgrown with *Phragmites australis*. Most of them are mesotrophic or eutrophic. The concentration of dissolved oxygen in the water is reduced to a minimum (0.2–0.5 mg/l) in winter with an increase in the thickness of ice to 0.8–1.7 m and the flow of biological processes without gas exchange at the boundary of the water and air. Hydrobionts are often dying out both in winter due to the prevalence of nitrification processes and in summer due to heat shock and lack of oxygen (Kitaev, 1984).

Despite severe habitat conditions, such lakes are typical habitats for *Gammarus lacustris*, a boreal-arctic species widespread in the Palearctic. This is facilitated by the wide tolerance of this species to temperature (from 0° to +30°C) and to various levels of dissolved oxygen in water. It dominates both in zoobenthos and among nektonic invertebrates. The amphipod has a mechanism for regulating internal homeostasis, which helps stabilize the level of metabolism during the annual cycle independent of temperature. Maintenance of homeostasis is based on the mechanism of osmoregulation of *G. lacustris*. In result, it is able to exist without gradual adaptation when a sharp increase of salinity to 30 g/l. Its populations in the lakes of the forest-steppe zone of Western Siberia form special faunistic complexes - a group of species adapted to certain abiotic and biotic habitats, due to the joint evolution and developing in one climatic zone, associated with certain types of landscapes and similar in their ecological characteristics (Nikolsky, 1947, 1953; Mordukhay-Boltovskoy, 1964).

More than 260 lakes in Western Siberia with a total area of about 400 km² are ecosystems with domination of *G. lacustris*. These lakes differ in the biomass of the gammarid populations. These values at the beginning of spring can range from 2.0 g/m²

to 264.0 g/m² (average 40.6 g/m²). By this time the populations are dominated by mature individuals, which account for 72.0% to 96.0% on abundance. The average size of individuals in *G. lacustris* populations varies from 10.7 to 15.6 mm with an average individual weight of 16 to 91 mg.

Gammarus lacustris is a species-edicator of lacustrine zoocenosis. In them, it can be both a detritophag and a predator using rotifers (*Keratella quadrata*), cladocera (*Daphnia magna*, *Daphnia pulex*, *Daphnia longispina*, *Daphnia obtusa*) and copepods (*Euritemora lacustris*, *Microcyclops gracilis*, *Eudiptomus graciloides*, *Eucyclops serrulatus*, *Cyclops strenuus*, *Acanthodiptomus denticornis*), chironomid larvae (*Chironomus plumosus*, *Limnochironomus nervosus*, *Cryptochironomus defectus*) and chaoborid larvae (*Chaoborus cristallinus* and *Chaoborus flavicans*), and in some cases weakened individuals of some fish species (*Carassius gibelio*, *Cyprinus carpio*). Gammarids are regulators of the population of planktonic crustaceans and of biodiversity in the ecosystems of the small lakes of the forest-steppe zone of the West Siberian Plain.

In lakes dominated by *G. lacustris*, phytoplankton is represented by typical species of blue-green algae (*Microcystis aeruginosa*, *Aphanizomenon flos-aquae*, *Anabaena flos-aquae*) and diatoms (*Asterionella formosa*). The phytoplankton was distributed in the spring relatively evenly in the water column and in June begins an increase of the algae biomass in the upper part of the photic zone with a decrease in the number of active zooplankton phytophages. The population density of algae was actively regulated by gammarids. The biomass of phytoplankton in the epilimnion varied in the ecosystems of such lakes within the range of 12–23 mg/l (wet weight) during the summer, of which an average of about 2–4% of the phytoplankton biomass on the size group of algae up to 20 µm - 34–38%, and more than 50 µm - 52–66%. Less than half of the biomass of phytoplankton accounted for algae accessible for consumption by zooplankton (cell size up to 50 µm). This value decreased to 20–30% during the intensive development of phytoplankton, which negatively influenced the development of zooplankton.

Up to 52–63% of the destruction of the plankton community in the investigated lakes was due to the activity of bacteria. Particularly high is the share of

bacterial destruction in the bottom layers, where the number of phyto- and zooplankton organisms is small. The intensity of destructive processes in lakes and the amount of detritus were in direct dependence (Rheinheimer, 2012).

The total number of bacterioplankton varied from 0.6 to 3.1 million cells/ml. The daily production of bacteria was low. The highest values were in August-September (0.7–0.9 million cells/ml). The ratio of the number of saprophytes to the total number of bacteria averaged 4–6%. Single cells accounted for 64–72% of the total number of bacteria, colonial forms - 12–20% and in the form of detritus-bacterial associations in water there were 9–16% of bacteria. The proportion of physiologically active cells was 79–83% of bacteria and at the time of the maximum number of bacteria decreased to 62%. The reason for this is that when the available food resource is exhausted, some of the bacteria go into a low-activity state.

The amount of seston changed during the growing season in the range of 82–115 g/m² (in terms of dry weight), reaching a maximum in mid-summer. Algae and bacteria accounted for about 38–43% of the seston, the rest - to the share of other plankton organisms and detritus. The amount of autochthonous dissolved organic matter (DOM) varied during the vegetation season within the range 56–82 gC/m². The concentration of DOM in the photic zone (depth up to 0.6 m) varied between 8 mgC/l and 10 mgC/l. The processes of excreting of biologically active substances by algae into the aquatic environment were most intensive in the early summer (in the lag-phase of development of the phytoplankton community) and late summer - early autumn during autoelimination of species in phytoplanktonocenosis (Findlay & Sinsabaugh, 2003).

Gammarus lacustris begins to predominate in the food of the cultivated *Coregonus peled* in such lakes only when the individuals of fish reach the mass of 20–24 g. This often occurs from the end of July or in August. The share of *Gammarus* in the diet of fish at this age stage reaches 90%. Young fish feed on more readily available cladocera (32% of the diet), copepods (17% of the diet) and algae. Adults of *G. lacustris* are not consumed by young fish because of their size and high activity in search of food. They themselves affect negatively on the fry of fish and sometimes act as predators.

CONCLUSIONS

Samples from 23 lakes of the forest-steppe zone of the West Siberian plain south-west part have been studied. The parasitofauna of gammarids is represented by five species of parasites (*Hymenolepis nana*, *Tetrameres fissispina*, *Streptocara crassicauda*, *Polymorphus magnus*, *Plagiorchis laricola*). An initial assessment of the occurrence of these species at different stages of their life cycle is provided. The work will continue in the future and will focus on biological safety of commercial products of gammarids and epizootic significance of parasites for the region.

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Update of checklist of marine Amphipoda in Tunisia from 2009 to April 2017

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ABSTRACT

In the present study, the list of the marine Amphipoda of Tunisian coasts was updated in the light of available data and new observations. We also include update of nomenclature. To establish this list, we reviewed all available records of Amphipoda since the last inventory of benthic marine amphipods. The number of Amphipoda species known on the Tunisian coasts was estimated now as 138 species belonging to 79 genera and 37 families.

KEY WORDS

Peracarida; checklist; Mediterranean Sea.

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INTRODUCTION

The Tunisian Amphipoda remained for long time unknown. In the earliest 20 century, Chevreux (1910, 1911) and Chevreux & Fage (1925) establish the first list of marine amphipods of Tunisian coasts with 65 species. New investigations were conducted from 2003 to 2009 and a new list was provided with 133 species belonging to 72 genera and 29 families (Zakhama-Sraieb et al., 2009). This study attempts to establish a complete update of all 138 marine amphipod species currently known in Tunisian coasts, including recent new records and species reported from the area.

NEW RECORDS

Ben Souissi et al. (2010) added three new records of non indigenous Amphipods, *Gammaropsis togensis* (Schellenberg, 1925), *Caprella scaura* Templeton, 1836 and *Hamimaera hamigera* (Haswell,

1880) in the Boughrara and El Bibane lagoon (South of Tunisia). *Caprella scaura* was also collected in the Tunis Southern lagoon between February 2012 and June 2015 (Ounifi Ben Amor et al., 2016). Mosbahi et al. (2015) reported two new records of Amphipoda in the Tunisian coast: *Lysianassa pilicornis* (Heller, 1866) and *Leucothoe incisa* (Robertson, 1892).

UPDATE OF NOMENCLATURE

We used the World Amphipoda Database to update the nomenclature of Tunisian Amphipoda. *Apolochus neapolitanus* (Della Valle, 1893) was reported as *Amphilochus neapolitanus* Della Valle, 1893 in Zakhama-Sraieb et al., 2009, *Nototropis guttatus* Costa, 1853 as *Atylus guttatus* (Costa, 1851), *Lysianassina longicornis* (Lucas, 1846) as *Lysianassa longicornis* (Lucas, 1846), *Lepidepcreum longicornis* (Spence Bate, 1862) as *Lepidepcreum longicorne* (Spence Bate &

Westwood, 1861), *Ceradocus* (*Ceradocus*) *orchestipes* Costa, 1853 as *Ceradocus orchestipes* Costa, 1853, *Deflexilodes griseus* (Della Valle, 1893) as *Monoculodes griseus* (Della Valle, 1893), *Parametaphoxus fultoni* (Scott, 1890) as *Metaphoxus fultoni* (Scott, 1890), *Cryptorchestia cavimana* Heller, 1865 as *Orchestia cavimana* Heller, 1865, *Orchestia gammarellus* (Pallas, 1766) as *Orchestia gammarella* (Pallas, 1766), *Britorchestia brito* (Stebbing, 1891) as *Talorchestia brito* Stebbing, 1891, *Deshayesorchestia deshayesii* (Audouin, 1826) as *Talorchestia deshayesii* (Audouin, 1826) and *Monocorophium insidiosum* (Crawford, 1937) as *Corophium insidiosum* Crawford, 1937.

CHECKLIST

SUB-ORDER GAMMARIDEA

FAMILY AMPELISCIDAE

- Ampelisca brevicornis* (Costa, 1853)
- Ampelisca diadema* (Costa, 1853)
- Ampelisca melitae* Dauvin et Bellan-Santini, 1985
- Ampelisca rubella* Costa, 1864
- Ampelisca serraticaudata* Chevreux, 1888
- Ampelisca spinipes* Boeck, 1861
- Ampelisca tenuicornis* Liljeborg, 1855
- Ampelisca typica* (Spence Bate, 1856)
- Ampelisca unidentata* (Schellenberg, 1936)

FAMILY AMPHILOCHIDAE

- Amphilochoides serratipes* (Norman, 1869)
- Apolochus neapolitanus* (Della Valle, 1893)

FAMILY ATYLIDAE

- Nototropis guttatus* Costa, 1853

FAMILY COLOMASTIGIDAE

- Colomastix pusilla* Grube, 1861

FAMILY CYPROIDEIDAE

- Peltocoxa marioni* Catta, 1875

FAMILY DEXAMINIDAE

- Dexamine spiniventris* (Costa, 1853)
- Dexamine spinosa* (Montagu, 1813)
- Guernea coalita* (Norman, 1868)
- Tritaeta gibbosa* (Spence Bate, 1862)

FAMILY IPHIMEDIIDAE

- Iphimedia minuta* G.O. Sars, 1882
- Iphimedia obesa* Rathke, 1843

FAMILY LEUCOTHOIDAE

- Leucothoe incisa* (Robertson, 1892)
- Leucothoe spinicarpa* (Abildgaard, 1789)
- Leucothoe venetiarum* Giordani-Soika, 1950

FAMILY LILJEBORGIIDAE

- Liljeborgia dellavallei* Stebbing, 1906

FAMILY LYSIANASSIDAE

- Hippomedon oculatus* Chevreux et Fage, 1925
- Lepidepecreum longicorne* (Spence Bate et Westwood, 1861)
- Lysianassa costae* Milne Edwards, 1830
- Lysianassa pilicornis* (Heller, 1866)
- Lysianassa plumosa* Boeck, 1871
- Lysianassina longicornis* (Lucas, 1846)
- Orchomene humilis* (Costa, 1853)
- Socarnes filicornis* (Heller, 1866)

FAMILY MEGALUROPIDAE

- Megaluropus massiliensis* Ledoyer, 1976
- Megaluropus monasteriensis* Ledoyer, 1976

FAMILY OEDICEROTIDAE

- Monoculodes carinatus* (Spence Bate, 1857)
- Deflexilodes griseus* (Della Valle, 1893)
- Perioculodes aequimanus* (Kossmann, 1880)
- Perioculodes longimanus longimanus* (Spence, Bate et Westwood, 1868)
- Pontocrates arenarius* (Bate, 1858)
- Westwoodilla rectirostris* (Della Valle, 1893)

FAMILY PHOXOCEPHALIDAE

- Parametaphoxus fultoni* (Scott, 1890)
- Metaphoxus simplex* (Spence Bate, 1857)
- Paraphoxus oculatus* (G.O. Sars, 1879)

FAMILY SCOPELOCHEIRIDAE

- Scopelocheirus hopei* (Costa, 1851).

FAMILY STEGOCEPHALIDAE

- Stegocephaloides christaniensis* Boeck, 1871

FAMILY STENOTHOIDAE

- Stenothoe bosporana* Sowinsky, 1898
- Stenothoe gallensis* Walker, 1904

- Stenothoe monoculoides* (Montagu, 1813)
Stenothoe tergestina (Nebeski, 1880)
- FAMILY SYNOPIIDAE
Pseudotiron bouvieri Chevreux, 1895
- FAMILY URISTIDAE
Ichnopus spinicornis Boeck, 1861
- FAMILY UROTHOIDAE
Urothoe elegans (Spencer Bate, 1857)
Urothoe pulchella (Costa, 1853)
- SUB-ORDER SENTICAUDATA
FAMILY AMPITHOIDAE
Ampithoe helleri Karaman, 1975
Ampithoe ramondi Audouin, 1826
Ampithoe riedli Schickel, 1968
Cymadusa crassicornis (Costa, 1857)
Cymadusa filosa (Savigny, 1816)
Sunamphitoe pelagica (H. Milne Edwards, 1830)
- FAMILY AORIDAE
Aora gracilis (Bate, 1857)
Aora spinicornis Afonso, 1976
Lembos websteri Spence Bate, 1857
Microdeutopus algicola Della Valle, 1893
Microdeutopus anomalus (Rathke, 1843)
Microdeutopus chelifera (Spence Bate, 1862)
Microdeutopus gryllotalpa Costa, 1853
Microdeutopus stationis Della Valle, 1893
Tethylembos viguieri (Chevreux, 1911)
- FAMILY BATHYPOREIIDAE
Bathyporeia nana Toulmond, 1966
- FAMILY CALLIOPIIDAE
Apherusa chierighinii Giordani-Soika, 1949
Apherusa mediterranea Chevreux, 1911
- FAMILY CAPRELLIDAE
Caprella acanthifera Leach, 1814
Caprella andreae Mayer, 1890
Caprella danilevskii Czerniavski, 1868
Caprella dilatata Krøyer 1843
Caprella equilibra Say, 1818
Caprella grandimana (Mayer, 1882)
Caprella hirsuta Mayer, 1890
Caprella liparotensis Haller, 1879
Caprella penantis Leach, 1814
- Caprella scaura* Templeton, 1836
Deutella schieckei Cavedini, 1982
Phtisica marina Slabber, 1769
Pseudoprotella phasma (Montagu, 1804)
- FAMILY CHEIROCRATIDAE
Cheirocratus assimilis (Liljeborg, 1851)
- FAMILY COROPHIIDAE
Apocorophium acutum (Chevreux, 1908)
Corophium orientale Schellenberg, 1928
Leptocheirus bispinosus Norman, 1908
Leptocheirus guttatus (Grube, 1864)
Leptocheirus pectinatus (Norman, 1869)
Leptocheirus pilosus Zaddach, 1844
Monocorophium acherusicum (Costa, 1853)
Monocorophium insidiosum (Crawford, 1937)
- FAMILY GAMMARIDAE
Echinogammarus olivii (H. Milne Edwards, 1830)
Gammarus aequicauda (Martynov, 1931)
Gammarus insensibilis Stock, 1966
Rhipidogammarus rhipidiophorus (Catta, 1878)
- FAMILY HYALIDAE
Hyale camptonyx (Heller, 1866)
Hyale grimaldii Chevreux, 1891
Hyale perieri (Lucas, 1849)
Hyale schmidtii (Heller, 1866)
Parhyale aquilina (Costa, 1857)
- FAMILY ISCHYRO CERIDAE
Ericthonius brasiliensis (Dana, 1855)
Ericthonius difformis Milne-Edwards, 1830
Ericthonius punctatus (Spence Bate, 1857)
Ischyrocerus inexpectatus Ruffo, 1959
Microjassa cumbrensis (Stebbing et Robertson, 1891)
- FAMILY MAERIDAE
Elasmopus brasiliensis (Dana, 1855)
Elasmopus pecteniscus (Spence Bate, 1862)
Elasmopus pocillimanus (Spence Bate, 1862)
Elasmopus rapax Costa, 1853
Hamimaera hamigera (Haswell, 1880)
Maera hirondellei Chevreux, 1900
Maerella tenuimana (Spence Bate, 1862)
Quadrimaera inaequipis Costa, 1857

FAMILY MELITIDAE

- Abludomelita aculeata* (Chevreux, 1911)
Ceradocus (Ceradocus) orchestiipes Costa, 1853
Melita palmata (Montagu, 1804)

FAMILY NUUANUIDAE

- Gammarella fucicola* (Leach, 1814)

FAMILY PHLIANTIDAE

- Pereionotus testudo* (Montagu, 1808)

FAMILY PHOTIDAE

- Gammaropsis dentata* Chevreux, 1900
Gammaropsis maculata (Johnston, 1827)
Gammaropsis ostroumowi (Sowinsky, 1898)
Gammaropsis togoensis (Schellenberg, 1925)
Gammaropsis ulrici Krapp-Schickel et Myers, 1979
Photis longipes (Della Valle, 1893)

FAMILY PODOCERIDAE

- Podocerus chelonophilus* (Chevreux et de Guerne, 1888)
Podocerus variegatus Leach, 1814

FAMILY PSEUDONIPHARGIDAE

- Pseudoniphargus africanus* Chevreux, 1901

FAMILY TALITRIDAE

- Cryptorchestia cavimana* Heller, 1865
Orchestia gammarellus (Pallas, 1766)
Orchestia mediterranea Costa, 1857
Orchestia montagui Audouin, 1826
Orchestia stephenseni Cecchini, 1928,

- Platorchestia platensis* (Krøyer, 1845)
Talitrus saltator (Montagu, 1808)
Britorchestia brito (Stebbing, 1891)
Deshayesorchestia deshayesii (Audouin, 1826)

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Hypogean amphipods from mountain springs on south Madagascar

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ABSTRACT

From the large freshwater system of Madagascar only few species of amphipods are known today. New material from springs and rivers in a mountainous area of south Madagascar has been studied and is preliminary placed in the Crangonyctoidea, and in the Madagascan endemic Allocrangonyctoidea.

KEY WORDS

Groundwater; Andringitra National Park; endemic species groups.

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In 2011, Reinhard Gerecke collected several amphipods from the Mount Ambatoberger spring area at an altitude of 2100 m. He donated them to Naturalis Biodiversity Center for study and some appear to be new species, for the time being placed in the Austroniphargidae Iannilli, Krapp & Ruffo, 2011. This family is known from Madagascar but not outside it. It is diagnosed by its coalesced urosomites (Fig. 1) and the lack of sternal gills. There are only ten freshwater amphipod species known since the description of *Austroniphargus bryophilus* (Monod, 1925). Seven are from springs and groundwater, and only three from surface waters. Recently they have been split into three families by Lowry & Myers, 2013, namely the Austroniphargidae *sensu stricto*, the Dussartiellidae, and the Sandroidae.

One of the new species (Fig. 1) is represented by nine specimens and provisionally placed in the parvorder Crangonyctidira. Diagnosis is as follows - Eyes absent, antenna 1 longer than antenna 2; accessory flagellum bi-articulate; first peduncle seg-

ment of antenna 2 enlarged and bulbous; lower lip with inner lobes; maxilla 1 with dissimilar palps, left palp with long, narrow setae, right palp with short, strong setae; gnathopods similar in size in male, gnathopod 2 slimmer in female, and with larger lobe on carpus than gnathopod 1; pereopod 3 and 4 subequal, pereopods 5–7 almost similar in size; pleopods 1–3 inner rami reduced to 1 long and 2 short articles; urosome coalesced; uropod 2 compact and stocky, endopod in male with apical hook-like robust setae; uropod 3 exopod elongated, endopod reduced to a small stump. Telson with concave posterior margin.

Apart from their interest as new species from an area that is a relatively white spot for crustacean research, the question of origin and relationship often comes to the front when dealing with Madagascan fauna. Lowry & Myers, 2013 commenting on the Madagascan freshwater amphipods, recognized Dussartiellidae as a clade separated from the sister taxa Austroniphargidae and Sandroidae. This analysis is corroborated for the moment by the geographic situation: the species in Dussartiellidae occur in the

Northern half of the island (*Reinhardia dimorpha* Iannilli, Krapp et Ruffo, 2011; *Dussartiella madagassa* Iannilli, Krapp et Ruffo, 2011; *Dussartiella aurifex* Iannilli, Krapp et Ruffo, 2011) while the species in Austroniphargidae (*Austroniphargus bryophilus*; *Libertinia latibasis* Iannilli, Krapp et Ruffo, 2011; *Libertinia longitelson* Iannilli, Krapp

et Ruffo, 2011; *Davidia spinicaudata* Iannilli, Krapp et Ruffo, 2011) and Sandoideae (*Sandro star-muehlneri* (Ruffo, 1960); *Sandro spinidactylus* Iannilli, Krapp et Ruffo, 2011) are found in the Southern half.

ACKNOWLEDGEMENTS

We thank Reinhard Gerecke for sending us his specimens, and University of Amsterdam MSc student Justin Vellinga for his work on the taxonomy.

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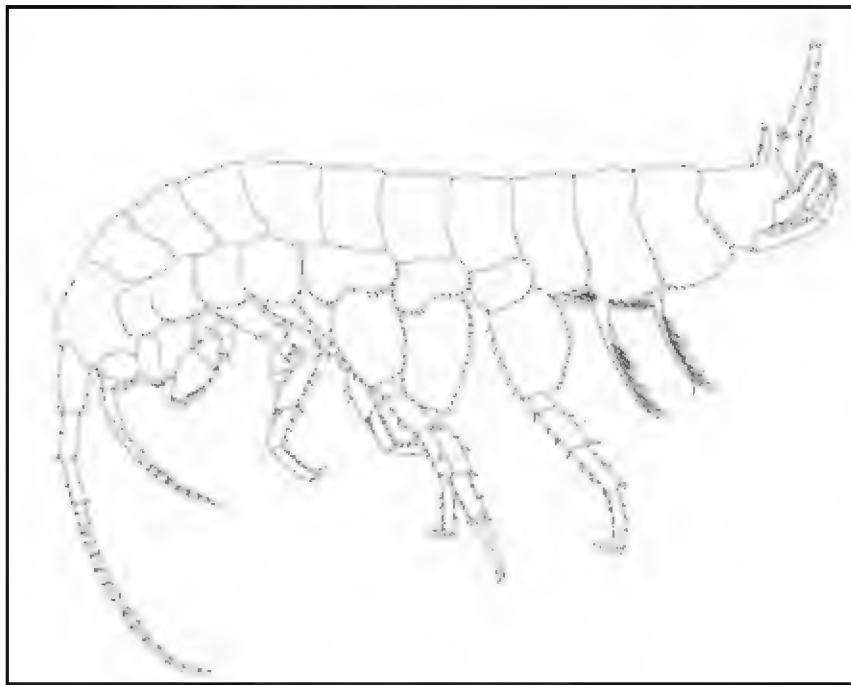


Figure 1. A male specimen, 5 mm, representing a new species in parvorder Crangonyctidira Lowry & Myers, 2013, collected at Mount Ambatoberger, spring area of Andringitra National Park (former province of Fianarantsoa), Madagascar (22°07'52.0" S, 046°53'30"; altitude 2105 m. coll. R. Gerecke, drawing J. Vellinga).

Tale of two rivers - long term study of Amphipoda species composition in the Vistula and the Oder rivers

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ABSTRACT

We presented changes in Amphipoda communities composition, occurring in two Polish rivers based on long term studies. Invasive species almost completely replaced natives.

KEY WORDS

Invasive species; distribution; spreading; Gammarids.

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INTRODUCTION

The Vistula (1047 km long) and Oder (866 km long) are among the longest and largest rivers of the Baltic Sea catchment area. Both are part of the so called Central Invasion Corridor commonly used by the Ponto-Caspian invasive aquatic hydrobionts. This route consists of the Dnieper river, Pripyat-Bug channel, connected first to the Baltic Sea by the Bug and Vistula rivers in Poland, and then to the North Sea basin via the Mittelland Channel in Germany. Industrial pollutions in the 19th century impoverished native fauna of those lowland large rivers and promoted the establishment of several alien, Ponto-Caspian and North-American amphipod species.

Our aim is to study the long-term dynamics of changes in species composition of the amphipod fauna inhabiting Vistula and Oder rivers, based on a 17-year-long study period. We also wanted to test if the two large rivers, different in their character and anthropogenic pressure (e.g. water transport), will differ also in the number of invasive species and dynamics of their invasions.

MATERIAL AND METHODS

Semi-quantitative kick-net samples were gathered in years 1998-2015 from the Vistula (10 dates, 14 sampling stations each date), and in years 2001–2015 from the Oder (7 dates, 12 sampling stations each date). To reveal the interactions among species, and the relations between species composition, water quality and conductivity were employed by the Kohonen artificial neural networks.

RESULTS

Among the nine amphipod species recorded in the Vistula river, we found six invasive Ponto-Caspian species: *Dikerogammarus villosus* (Sowinsky, 1894), *D. haemobaphes* (Eichwald, 1841), *Echinogammarus ischnus* (Stebbing, 1899), *Pontogammarus robustoides* (G.O. Sars, 1894), *Obesogammarus crassus* (G.O. Sars, 1894) and *Chelicorophium curvispinum* (G.O. Sars, 1895); one North-America invasive species - *Gammarus tigrinus* Sexton, 1939, and one species of Balkan origin - *Gammarus roe-*

selii Gervais, 1835. Only one native species, *Gammarus fossarum*, was detected in this river. Also nine species were found in the Oder river, but with some distinctions: *E. ischnus* and *G. fossarum* Koch, in Panzer, 1836, were absent and instead we found *Echinogammarus trichiatus* (Martynov, 1932) and *Gammarus lacustris* G.O. Sars, 1863. Given that one Ponto-Caspian and one native species were swapped, seems that the composition of the community was the same based on the observed data.

CONCLUSIONS

Preliminary results showed almost complete exclusion of native gammarids in the Vistula and in the Oder rivers. Single localities with native species were observed in the Vistula in some years, whilst only one individual of *Gammarus fossarum* was found in the Oder during the 14 years of study. The most probable scenario is extinction of gammarids in large rivers during the past years of the 20th century, caused by industrial pollution. Invasive species with wider ecological tolerance replaced them. Now, even after the improvement of water quality, the native amphipods did not return, probably due to high competition from the already well-established and abundant populations of invasive spe-

cies. On the other hand, all native species survived well in smaller tributaries. Concerning the composition of the invasive species communities, we can observe the clear pattern that *D. haemobaphes* has appeared earlier and spread fast in both rivers herein analyzed when compared with *D. villous*. However, when the latter species appeared (1999, first record in Oder and 2007, first record in Vistula), it has quickly outnumbered the former one. We also observed that *G. tigrinus* has occupied the parts of the rivers with high water conductivity. The mysterious appearance of this species in upper Vistula in 2011, could be caused by local flood in Upper Oder drainage, and its temporary connection to the Vistula drainage. This would be one of the few cases where the spreading of invasive species was enhanced by natural disasters.

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Stoichiometric differences between invasive *Pontogammarus robustoides* (Sars, 1894) (Pontogammaridae) and local *Gammarus lacustris* G.O. Sars, 1864 (Gammaridae)

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ABSTRACT Stoichiometric traits of invasive *Pontogammarus robustoides* (Sars, 1864) (Pontogammaridae) and local *Gammarus lacustris* (G.O. Sars, 1894) (Gammaridae) juveniles were explored in a laboratory experiment

KEY WORDS *Gammarus lacustris*; *Pontogammarus robustoides*; stoichiometry.

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Laboratory-acclimated gravid *Gammarus lacustris* G.O. Sars, 1864 (Gammaridae) and *Pontogammarus robustoides* (Sars, 1894) (Pontogammaridae) females were divided into equal groups (4 groups for each species). Mothers were restricted within net cages to protect the young from predation, and accommodated in separate aquariums in recirculatory system. Outflow pipes were covered with filters to stop amphipods from mixing. The mothers were removed and saved for analysis after all juveniles hatched. Before the experiment all females were fed the same crustacean food, while during experiment they were fed the experimental food.

Two kinds of experimental food were used - dried filamentous algae (carbon, nitrogen and phosphorus ratio C/N/P = 338.94/28.92/1) and dried chironomid larvae (C/N/P = 240.04/36.76/1). There were two aquariums for each food-species combination. The juvenile sampling was performed after 30 and 60 days after hatching of the last juveniles.

Percentages of C and N in the samples were determined using continuous-flow isotope mass spectrometry, whereas P content was estimated using a molybdate-ascorbic acid method. The results were analysed using ANOVA.

Although chironomid larvae are richer in nutrients, no statistically significant effect of food qual-

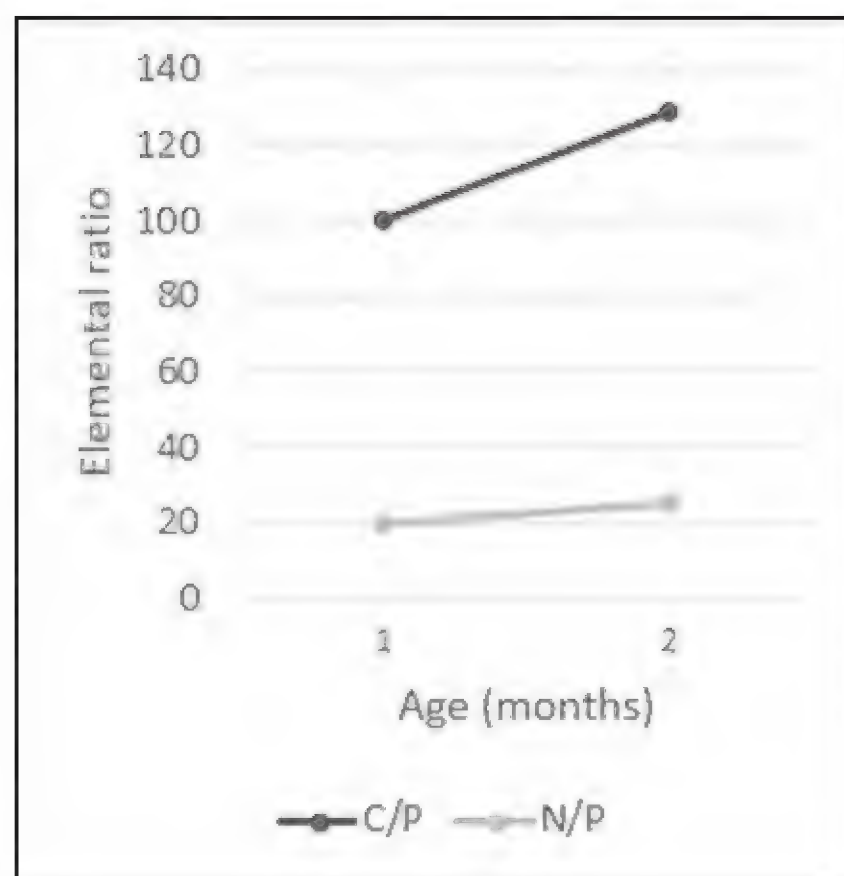


Figure 1. Difference in carbon/phosphorus and nitrogen/phosphorus ratios (C/P and N/P) between one and two months old amphipod juveniles (both species combined).

ity on body stoichiometry was detected. It proves that both species demonstrate strict homeostasis.

It was found that P content, C/P and N/P depended on age and species. Both C/P and N/P increased with age, showing decreasing P percentage in the organism (ANOVA: C/P $p=0.018$, N/P $p=0.007$) (Fig. 1). This is consistent with growth rate hypothesis, which states that P content correlates with organism's growth rate.

Interestingly, *G. lacustris* demonstrated lower C/P and N/P ratios than invasive *P. robustoides* (ANOVA: C/P $p=0.015$, N/P $p=0.011$) (Fig. 2). It was expected that the invader that was outcompeting the local species had a higher growth rate, but these results would suggest otherwise.

No C/P or N/P differences were detected between mother amphipods of different species, but *G. lacustris* had a higher C/N ratio ($p=0.0086$). The lack of C/P and N/P difference in adults supports the assumption that the difference in juveniles was related to growth rate. However, it is possible that *P. robustoides* has higher requirements for food (due to physiological or anatomical differences) and

could not reach their maximum growth rate in our experimental conditions.

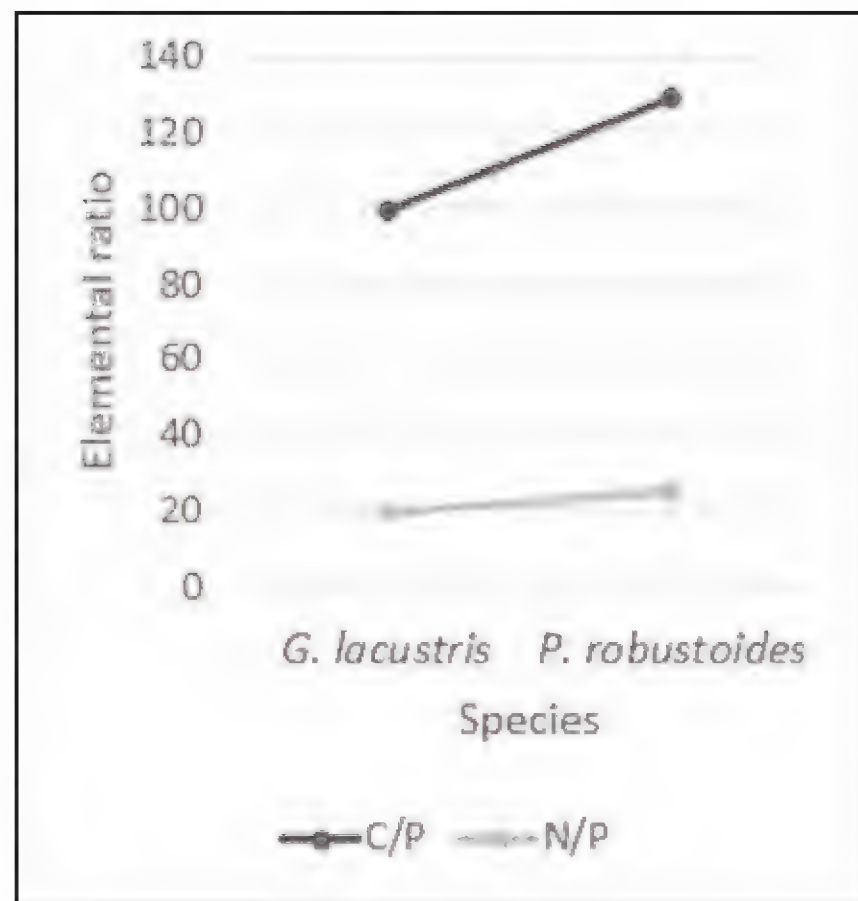


Figure 2. Difference in carbon/phosphorus and nitrogen/phosphorus ratios (C/P and N/P) between *G. lacustris* and *P. robustoides* juveniles (both ages combined).

Abyssal amphipods from the Vema Fracture Zone (tropical N Atlantic): diversity and abundance at both sides of the Mid Atlantic Ridge

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ABSTRACT

The composition of the amphipod fauna along an E-W transect in the Vema Fracture Zone (tropical N Atlantic) is analyzed in order to examine whether the fracture allows its distribution across the Atlantic Ocean.

KEY WORDS

Abyssal amphipods; Vema Fracture; Mid-Atlantic Ridge; N Atlantic Ocean.

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INTRODUCTION

The abyssal seafloor makes up > 60% of our planet's surface, it is nevertheless largely unexplored. In the Atlantic, transform faults and fracture zones characterize most of the seafloor bathymetry and the volcanic and tectonic processes which create and modify the crust can be deduced from their bathymetric signature. During the Vema-TRANSIT expedition, one of the major offsets of the Mid-Atlantic Ridge (MAR), the Vema Fracture Zone (VFZ), was sampled in its entire length (Fig. 1). Variations in benthic communities along this transect were investigated to test the hypothesis that the MAR serves as a barrier limiting species distribution in the abyssal basins on both sides of the ridge (Brandt et al., 2015; Devey, 2015).

MATERIAL AND METHODS

Within the framework of the German multi-disciplinary Vema-TRANSIT project, the suprabenthic fauna of the VFZ was sampled using a camera-

epibenthic sledge (Brandt et al., 2013). This gear is equipped with two superimposed nets (0.5 mm mesh size, 0.3 mm at the codend) and samples the motile fauna in the 25–60 and 77–112 cm water layers above the seafloor. Sledge samplings were carried out during December 2014 and January 2015 at 11 stations located between 4986 and 5735 m depth (Fig. 1).

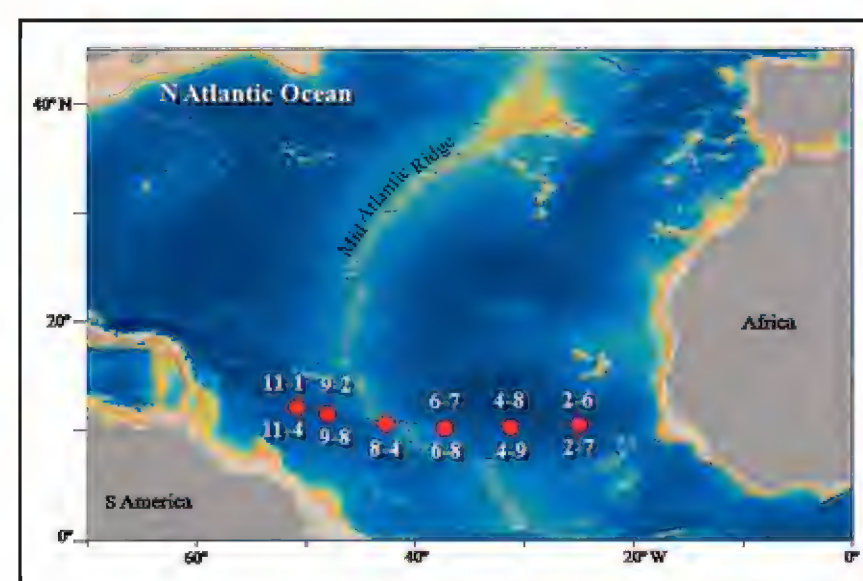


Figure 1. Sampling stations in the Vema Fracture Zone during Vema-TRANSIT expedition.

RESULTS AND CONCLUSIONS

Overall, 8.4% of the perarid fauna collected at the VFZ were amphipods. A total of 348 individuals belonging to at least 50 species were identified. The suborder Gammaridea (98% of the total amphipods) was the best represented, but Senticaudata and Hyperiidea occurred as well (1.7 and 0.3%, respectively). In the twenty families reported, a dominance of the typical deep-sea families were observed: Phoxocephalidae (24.1% of the total amphipods), Pardaliscidae (22.7%), Oedicerotidae (12.9%), Eusiridae (8.9%), Stenothoidae (8.0%) and Synopiidae (7.5%) (Fig. 2). Pardaliscidae was the most frequent, occurring in ten stations. Stegocephalidae, Stilipedidae, Vitjazianidae and Hyperidae were recorded only at the eastern abyssal plain, whereas Lepechinellidae only occurred in the western one and Ampeliscidae in the MAR. The total abundance ranged between 4.2 and 33.8 ind./1000 m₂ (St 11–4 and 9–8, respectively), but in general higher values were shown in the eastern VFZ than in the western one. Furthermore, the species turnover along the East to West transect will help us to understand the deep-sea latitudinal gradients of the species in the Atlantic Ocean.

ACKNOWLEDGEMENTS

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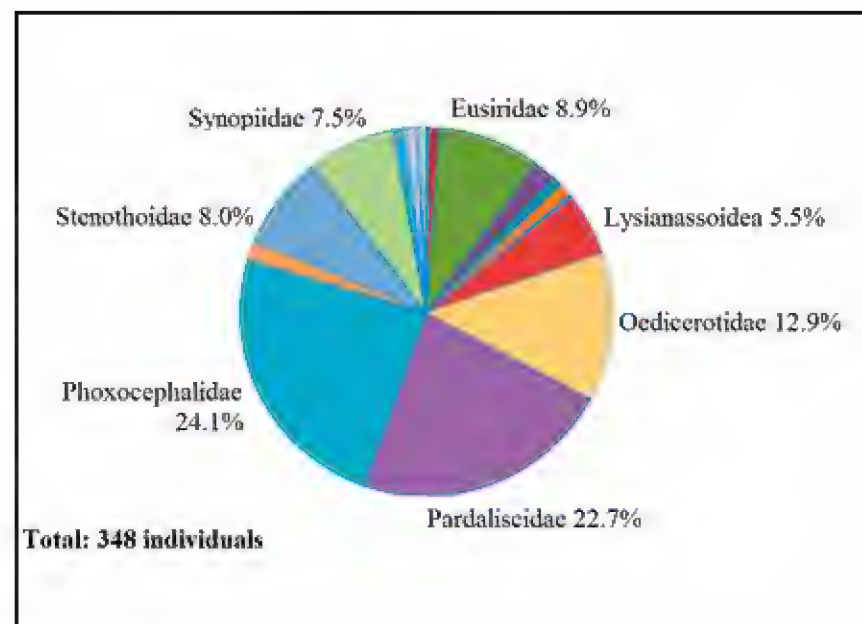


Figure 2. Global composition of Amphipoda in the Vema Fracture Zone during Vema-TRANSIT expedition.

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Diversity of deep-sea Amphipoda from Papua New Guinea (SW Pacific Ocean)

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ABSTRACT

New insights into the diversity of deep-sea amphipods in the Papua New Guinea seas are provided as result of the Tropical Deep-Sea Benthos program.

KEY WORDS

Deep-sea amphipods; diversity; Papua New Guinea; SW Pacific.

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INTRODUCTION

The island of New Guinea has a privileged place in the heart of biologists. In that tropical area, terrestrial biodiversity studies have helped shape some of the pillars of evolutionary biology and ecological theory (Wallace, 1869, 1876; Mayr, 1942). Contrasting with our knowledge on these terrestrial ecosystems is the lack of information on adjacent deep-sea ecosystems. The pioneering Challenger and Siboga deep-sea expedition of the 19th century, devoted little attention to the sampling of New Guinea marine waters. Later on, only the Galathea and Vityaz expeditions carried out some benthic samplings in that area.

The geological complexity and the rapid changes observed in the Papua New Guinea (PNG) region are of particular biogeographic interest. The Bismarck and Solomon seas host many underwater features such as seamounts or hydrothermal vents. Significant efforts for describing the hydrothermal vent-endemic fauna have been made (Desbruyères et al., 2006), but the general deep benthic fauna is yet to be described: only 34 deep-water crustacean

species are listed in OBIS database (Van den Berghe, 2007).

The Tropical Deep-Sea Benthos (TDSB) program, organized by both the Muséum National d'Histoire Naturelle and the Institut de Recherche pour le Développement French institutions, is devoted to fill in the gap in our knowledge of deep-sea biodiversity (Bouchet et al., 2008; Richer de Forges et al., 2013). Exploring PNG seas has represented a challenge for the TDSB program and since 2010, four deep-sea surveys were organized in Bismarck and Solomon seas (Biopapua, 2010; Papua Niugini, 2012; Madeep & Kavieng, 2014) (Fig. 1).

The sampling methods are based on bottom-towed devices: the Warén dredge (deployed on rocky bottoms) and the French beam trawl (deployed on soft bottoms). The cod-end of the last one has an inner smaller mesh-sized bag favouring the collect of small peracarids such as amphipods.

RESULTS AND CONCLUSIONS

Although dredge and trawl surveys were not

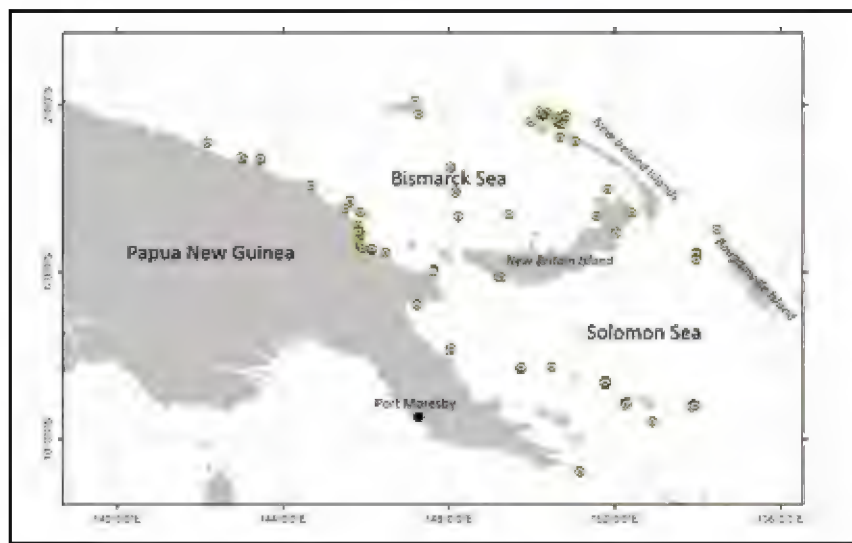


Figure 1. Sampling stations visited during Biopapua, Papua Niugini, Madeep and Kavieng surveys.

specifically dedicated to the sampling of peracarids, a total of 755 amphipods has been collected during the PNG deep-sea cruises, at 100 sampling stations ranging between 120 and 1252 m depth (Fig. 1). This collected material is still under study.

At the present stage, the diversity of this amphipod collection is estimated to more than 78 species belonging to at least 32 families, represented by 59 species for Gammaridea (75.6%), 8 species for Hyperiidea (10.3%) and 11 species for Senticaudata (14.1%). The percentage of new species is more than 20%, belonging to genera *Epimeria*, *Adeliella*, *Rhachotropis*, *Valettropsis*, *Parargissa*, *Syrrhoe*, *Euonyx*, *Ichnopus*, *Stephonyx*. The main families are Cheluridae (27.2% of total abundance; depth: 120–219 m), Epimeriidae (22.3%; 306–1912 m), Pontogeneiidae (19.2%; 306–808 m), Calliopidae (4.2%; 440–960 m), Stegocephalidae (3.8%; 306–700 m), Uristidae (2.6%; 275–855 m), Lysianassoidea *incertae sedis* (2.6%; 260–1045 m) and Eusiridae (2.5%; 340–998 m). The Cheluriidae are represented by only one species (new to Science) of the genus *Tropichelura*, mainly dominant on the

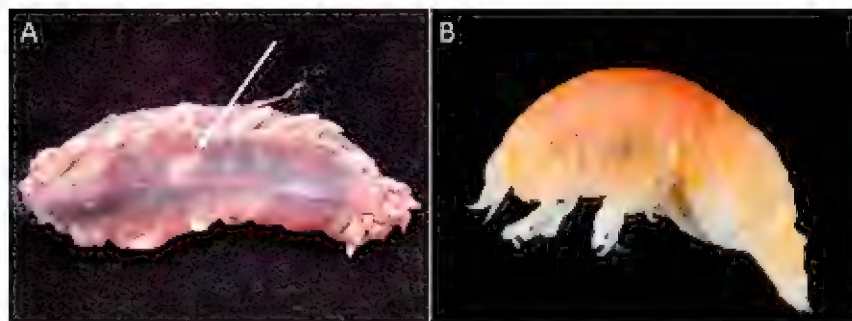


Figure 2. A) *Adeliella* sp. amphipod seen through the integuments of a sea cucumber; photo after specimen collection (A. Warén). B) *Adeliella* sp. free collected in the Solomon Sea; photo after ethanol preservation (I. Frutos).

outer shelf of the Bismarck Sea. The Lysianassoidea *incertae sedis* is also a new species, belonging to genus *Adeliella* (Fig. 2B). Most specimens of the latest new species were collected free in beam-trawl samples from Bismarck and Solomon seas. However, some of them were housed in the coelomic cavity of elasipod holothurians, as evidenced by the figure 2A.

ACKNOWLEDGEMENTS

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Full mitogenome of *Dikerogammarus villosus* (Sowinsky, 1894) (Gammaridae) and a glimpse of the Amphipoda mitogenome phylogenetics

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ABSTRACT

Using High-Throughput Sequencing full mitogenome of *Dikerogammarus villosus* (Sowinsky, 1894) (Gammaridae) and partial mitogenomes of other Ponto-Caspian Amphipoda species were obtained and analyzed.

KEY WORDS

Mitogenome; *Dikerogammarus villosus*; High-Throughput Sequencing; Phylogenetics.

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INTRODUCTION

The amphipod *Dikerogammarus villosus* has colonized most of the European main inland water bodies in less than 20 years, having deteriorating effect on the local benthic communities. The evolutionary processes behind invasion of this species are still being under examination by many scientists. The aim of our studies was to provide the full mitogenome of *D. villosus* as well as of other Ponto-Caspian invasive gammarids in order to help to clarify the taxonomy of the group and to provide a reference library for future metabarcoding and evolutionary studies in Amphipoda.

MATERIAL AND METHODS

We have performed the High-Throughput Se-

quencing (HTS) using Illumina MiSeq of the total DNA from the samples of *D. villosus* from the Western Invasion Route (Oder River in Poland) and from the native range (Terkos Lake in Turkey). Additionally we have performed HTS on samples of *Pontogammarus robustoides* and *D. haemobaphes* from Poland. The phylogeny was reconstructed with Bayesian inference based on the acquired data and other mitogenomes already available for Amphipoda.

RESULTS AND CONCLUSIONS

The sequencing results allowed us to reconstruct the full mitogenome of *D. villosus* and partial mitogenome sequences in case of the other Ponto-Caspian species. The reconstructed Bayesian tree suggests that the approach incorporating mitogen-

omes can help in obtaining well resolved phylogenies for amphipods, especially in the case of a fam-

ily whose taxonomy is highly disputable, such as Gammaridae.

Compiling a global DNA barcode reference library for marine amphipods (Crustacea Amphipoda)

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ABSTRACT

This study consists on a compilation, auditing, annotation and analysis of a global reference library of DNA barcodes for marine amphipods, based on all current and publicly available DNA barcodes in BOLD. We found eligible DNA barcodes for only 223 amphipod species, among which 18% comprised multiple intraspecific lineages displaying comparatively high divergence. A substantial research effort is still required to build up the global DNA barcode reference library for marine amphipods.

KEY WORDS

Amphipoda; cytochrome c oxidase subunit I; DNA barcode reference library; taxonomy.

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INTRODUCTION

Amphipods are one of the most diverse and widely distributed groups of crustaceans. In marine habitats, amphipod species are among the most ecologically important invertebrates, with high relevance in trophic interactions and constituting dominant groups and key components of marine benthic communities (Ruffo, 1998). This order currently contains more than 9,000 known species listed in the World Amphipoda Database (Horton et al., 2017), and there is no doubt that numerous species await formal description. As for many other marine invertebrate taxa, rigorous species identifications can be rather challenging, with the knowledge of amphipod diversity being constrained by the taxonomic impediment. Specific features of amphipods, such as sexual dimorphism and morphol-

ogical similarity among closely related species, limit our ability to discriminate species based only on morphological characters (Lobo et al., 2016).

After the generalization of the use of DNA barcodes as a universal system for DNA-based species identification, DNA metabarcoding and environmental DNA (eDNA) monitoring are emerging as central components of the new generation of bio-monitoring systems (Leese et al., 2016). However, the availability of reliable, scrutinized and annotated reference libraries of DNA barcodes is a fundamental backbone of these DNA-based approaches (Raupach & Radulovici, 2015).

MATERIAL AND METHODS

The objective of this study was to compile,

audit, annotate and analyse a global reference library of DNA barcodes for marine amphipods, based on all current and publicly available DNA barcodes in BOLD.

A dataset was created on Barcode of Life Data Systems (BOLD), including new and previously generated sequences by the research groups authoring the current manuscript, encompassing samples from the Atlantic Ocean, Mediterranean Sea, North Sea and Baltic Sea. Additionally, representative sequences of all amphipod species publicly available on BOLD from marine environments across the world on 15/04/2017 were added. Only barcodes with a minimum length of 500 bp, without contamination or stop codons, and with indication of sampling location and taxonomic identification, were used.

All sequence edition and genetic analyses were performed using the software MEGA 7.0 (Kumar et al., 2016) and the BOLD analyses tools (Ratnasingham & Hebert, 2013). Sequences were aligned using Muscle (Edgar, 2004) and the Barcode Index Number (BIN) system (Ratnasingham & Hebert, 2013) was used as delimitation criterion for assignment of molecular operational taxonomic units (MOTU) across the full dataset. To help visualizing the partitioning of sequences according to their similarity, a Neighbor-Joining phenogram was constructed applying the Kimura 2-parameter (K2P) substitution model (Kimura, 1980) and using 1,000 bootstrap iterations to determine the node's support. Intra and interspecific genetic distances were calculated using K2P implemented in the distance summary tool in BOLD, to facilitate comparison with other studies.

RESULTS

A search in BOLD Systems v4 on 15/04/2017 retrieved 18,506 public sequences from 1,177 amphipod species. Only 1,849 sequences pertained to marine species and achieved the pre-established quality criteria, but a large number of published DNA barcodes may have been left out because they did not have associated GPS coordinates. These barcodes belong to 223 species and 59 families, with the families Talitridae (438 barcodes), Gammaridae (249 barcodes) and Caprellidae (203 barcodes) being the most well represented. Iberian Peninsula (456 barcodes), Canada (324 barcodes) and North Sea (303 barcodes) were the best repre-

sented regions and the North Atlantic Ocean comprised most of the sequences (84%), while no amphipod barcodes were publicly available from the Indian Ocean. These 223 amphipod species were assigned to 293 distinct BINs, 6 being discordant, and 287 concordant (102 singletons). As much as 18% of the species comprised multiple lineages with comparatively high divergences. The species *Dexamine spinosa* (24.06%), *Ampithoe rubricata* (24.76%) and *Apothyale stebbingi* (23.94%) displayed the highest intraspecific distances.

CONCLUSIONS

Although the order Amphipoda is diverse and abundant across world marine environments, only 223 of the 8,113 known marine amphipod species (2.5%) are currently represented in BOLD by records fulfilling our selection criteria. Moreover, there is a large gap of representative species barcodes from most oceans, with the Atlantic Ocean disproportionately represented with 84% of the public barcodes. The high level of hidden diversity disclosed in this study confirms the importance of integrating data from multiple sources, in order to unravel pertinent cases of taxonomic uncertainties that otherwise would have remained unnoticed. A substantial research effort is still required to further populate the DNA barcode reference library, in order to benefit from the full potential of DNA-based biomonitoring tools and deepen our understanding of the diversity, distribution and ecology of marine Amphipoda.

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A preliminary assessment of the amphipod collections in Naturalistic Museums in Italy

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ABSTRACT A first assessment on the presence of Amphipoda collections in Italian Natural History Museums is given.

KEY WORDS Amphipoda; Natural History Museum collections; Italy.

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INTRODUCTION

With the exception of the Museo Civico di Storia Naturale in Verona, where the precious and huge collection of Amphipoda studied and preserved by Sandro Ruffo is present (Ruffo & Krapp, 2005), few information are available regarding the presence of specimens belonging to this order in Italian Museums of Natural History.

For instance, a year ago, just by chance, it was possible to ascertain the presence of Clelia Cecchini's amphipod type specimens in the Museo di Storia Naturale, Università di Firenze (Lo Brutto, 2017).

Thus, a first survey on the presence of amphipods among the Arthropoda Crustacea collections has been carried out, with the aid of the Associazione Nazionale Musei Scientifici (ANMS; www.anms.it) database, in order to give a preliminary evaluation to the researchers in the field.

RESULTS AND CONCLUSIONS

Thirty-three Museums, belonging to different Institutions (Municipality, University or other) were

contacted by e-mail. Museums confirming the presence of amphipods were later on contacted in order to have as much information as possible, regarding the consistence of the collection, the origin, the researchers who studied them and if type specimens were among the material. Only 11 Museums confirmed to held Amphipoda among the Crustacean collections, 3 declared that the collections were present, but their status could not be assessed, while 2 Museums never answered (Table 1).

Among the above-mentioned 11 Museums, only five (Bergamo, Ferrara, Firenze, Genova and Udine) detain conspicuous collections, that is to say, more than 100 specimens, comprising type specimens, from both freshwater and marine habitats, mostly from Italian rivers, caves, lagoons and seas. Indeed the majority of the amphipods held in the Italian Museums are from freshwater habitats (Fig. 1), with the collection in Udine Museum reaching more than 47,000 specimens (P. Glerean, pers. comm.) collected from the Friuli region.

Prof. Sandro Ruffo and Dr Fabio Stoch have identified most of them. Historical and recent new species were studied by Clelia Cecchini (Firenze), Teresita Maccagno (Genova) and Cristina Munari (Ferrara).

Museums holding Amphipoda collections	City	Type specimens present
Museo Civico di Scienze Naturali "E. Caffi"	Bergamo	
Museo Civico di Storia Naturale	Carmagnola (Torino)	
Museo Civico di Storia Naturale	Ferrara	x
Museo di Storia Naturale, Università di Firenze	Firenze	x
Museo Civico di Storia Naturale "G. Doria"	Genova	x
Stazione Zoologica Anton Dohrn	Napoli	
Museo di Storia naturale, Università di Parma	Parma	
Accademia dei Fisiocritici	Siena	
Museo delle Scienze di Trento	Trento	
Musei Civici di Storia Naturale di Trieste	Trieste	
Museo Friulano di Storia Naturale	Udine	x
Sistema museale di ateneo, Università di Pavia	Pavia	NAV
Museo Regionale di Scienze naturali	Torino	NAV
Museo Civico di Storia Naturale	Venezia	NAV
Civici musei e gallerie	Reggio Emilia	NA
Museo di Storia naturale dello stretto di Messina e del Mediterraneo	Villa San Giovanni (Reggio Calabria)	NA
NAV=Not Available; NA=No Answer		

Table 1. Lists of the contacted Italian Museums holding Amphipoda, with unavailable collections or without information.

However, all the Museums are preserving many specimens, from several Italian localities, still to be studied and identified.

This preliminary assessment on the Italian Natural History Museum collections gives a first glance on the presence of this group. Italian Museums, as archives of biodiversity, are preserving a

relevant quantity of Amphipoda, mostly from Italian localities, many of them available for study to interested researchers.

ACKNOWLEDGEMENTS

The author is grateful to Dr Sabrina Lo Brutto (University of Palermo, Italy) for spurring the preparation of this note; many thanks are due to all the Museum curators that answered this survey.

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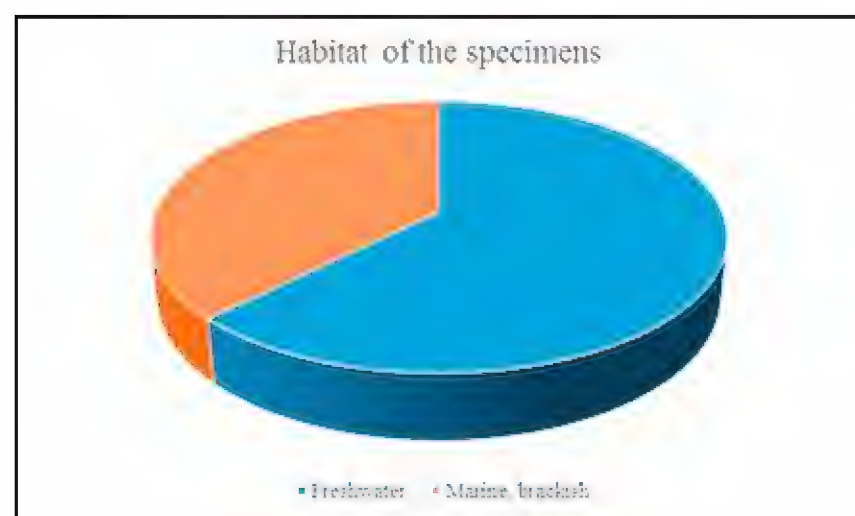


Figure 1. Data on the origin habitat of the Amphipoda collections in Italian Museums (orange: marine or brackish; blue: freshwater).

Diversity and origin of freshwater gammarids from Sicily (Italy): preliminary results

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ABSTRACT

We have revealed presence of two morphospecies on Sicily (Italy) with extremely high level of cryptic diversity. Multi-marker phylogeny revealed different origin for these species.

KEY WORDS

Amphipoda, Sicily, origin, phylogeny, phylogeography

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INTRODUCTION

The Mediterranean Region is known as one of the most prominent biodiversity hotspots worldwide. However, majority of studies upon Gammaridae of the region focused mostly on marine species, leaving aside the freshwater fauna. So far, around 120 freshwater species of two genera, *Gammarus* Fabricius, 1775 and *Echinogammarus* Stebbing, 1899, have been reported from the area, with only 15 known from the Mediterranean islands. Given the very high cryptic diversity discovered recently in European gammarids and relative scarcity of studies upon insular species, we conclude that number of species already reported both from the mainland and from these islands may be underestimated.

Our main goal is to reveal the phylogenetic relationships and origin of freshwater gammarids inhabiting Sicily, a large Mediterranean island of continental origin. Our additional goal is to estimate the biodiversity of members of *Echinogammarus* in the Apennine Peninsula and Tunisia, which serves as the reference regions, which used to have land connection with the studied island.

MATERIAL AND METHODS

A set of 178 individuals collected in 2016 from 28 locations in central and southern part of the Apennine Peninsula and Sicily supplemented by 126 specimens from 17 locations collected from Tunisia in 2011-2015 were amplified for COI (557 bp) as well as other molecular markers like 16S rRNA (356 bp) and nuclear ITS-1 (665 bp) and 28S rRNA (1163 bp). MOTUs were delimited using four different methods: ABGD, GMYC (single- and multiple-threshold models) and PTP. The selection of appropriate substitution model was done in bModel test (a package for BEAUti) and reconstruction of time-calibrated phylogeny was done using BEAST software package.

RESULTS

We have revealed presence of two morphospecies on Sicily, which are already known members of the genus *Echinogammarus*: *E. sicilianus* and *E. adipatus*. The former was present only in the

eastern part of Sicily, whereas the latter was present also in the western part. The two morphospecies coexisted only at one sampling site. Both were monophyletic and exhibited substantial level of cryptic diversity. We identified three MOTUs in case of *E. adipatus* (5.0–9.5% K2P) and nine MOTUs in case of *E. sicilianus* (6.0–24.5% K2P), confirmed by all the applied delimitation methods. The reconstruction of phylogeny showed that *E. adipatus* is most closely related to *E. cf. pungens* from Tunisia and their divergence started probably ca. 11 million years ago. On the other hand *E. sicilianus* is sister group to all *Echinogammarus* from Italy, from which it diverged probably ca. 25 mln years ago. Additionally, we revealed presence of at least thirteen MOTUs of *Echinogammarus* on the Apennine Peninsula and at least twelve in Tunisia.

CONCLUSIONS

Preliminary results of phylogeny reconstructed

with several both mitochondrial (COI and 16S) and nuclear (28S and ITS-1) markers revealed African origin for *E. adipatus* and its divergence from the Tunisian *E. cf. pungens* probably happened in late Serravallian/early Tortonian. Around this time, part of land which now is Sicily separated from the North-African plate. This indicates possibly continental origin for this species. On the other side *E. sicilianus* diverged from the Apennine *Echinogammarus* around late Oligocene/early Miocene and around that time the Mediterranean-Paratethys seaway through the Alps was re-opened and one through Slovenia was closed, so it may be an indication for its marine origin. Additionally *Echinogammarus* members from the Apennine Peninsula and Tunisia shown high cryptic diversity, largely exceeding the number of morphospecies described from these areas. This shows a similar pattern to the one identified recently in the genus *Gammarus*.

Is the Mediterranean Sea a hotspot for cryptic species? The case of two widespread brackishwater *Gammarus* Fabricius, 1775 species (Crustacea Amphipoda)

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ABSTRACT

We have detected cryptic diversity in two brackishwater *Gammarus* Fabricius, 1775 species. Multi-marker time-calibrated phylogeny was reconstructed for *G. aequicauda* (Martynov, 1931).

KEY WORDS

Brackishwater; *Gammarus*; Amphipoda; phylogeny; cryptic .

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INTRODUCTION

Gammarid amphipods are among the most abundant members of macroinvertebrate communities in freshwaters as well as marine and brackish waters of the basins of the Mediterranean Sea, the Black Sea and the North Atlantic. They are widely used in numerous ecotoxicological studies and bio-monitoring assessments. Most recent studies have proved extremely high level of cryptic diversity for several members of *Gammarus* Fabricius, 1775 inhabiting freshwater bodies from numerous locations in Europe. Despite the members of the so-called “*Gammarus locusta* group” are widespread in brackish habitats of the Mediterranean Sea and the Black Sea, no detailed molecular assessment has yet been made for that group, including *G. aequicauda* (Martynov, 1931) and *G. insensibilis* Stock, 1966

which are the most frequent and widely distributed *Gammarus* representatives in those basins. The goal of our study was to investigate the diversity and the phylogenetic relations of two members of the *G. locusta* (Linnaeus, 1758) group, *G. aequicauda* and *G. insensibilis*, over a wide distribution area.

MATERIAL AND METHODS

Partial DNA sequences (510 bp) of the mitochondrial cytochrome oxidase subunit I gene (COI) were obtained and compiled for a collection of 212 individuals of *G. aequicauda*, *G. insensibilis*, *G. crinicornis* Stock, 1966 and *G. locusta* from 63 locations along the European Atlantic coast between Germany and Portugal, and from the Mediterranean Sea and the Black Sea. Molecular operational tax-

onomic units (MOTUs) were delimited using five different methods: ABGD, GMYC (single- and multiple-threshold models), PTP and BINs. Representative specimens of each of the most consistent MOTUs derived from COI data were then sequenced for one additional mitochondrial region (16S) and two nuclear loci (28S and ITS-1) in order to reconstruct a time-calibrated phylogeny. The software package BEAST was used to select the best-fit substitution model and to perform the time-calibrated phylogenetic reconstruction.

RESULTS

The deep divergence between Black Sea, Mediterranean Sea and Atlantic populations in both *G. aequicauda* and *G. insensibilis* was supported by all five MOTU delimitation methods. There were 4-8 distinct MOTUs delimited for *G. aequicauda* (3.0-14.0% K2P) and 3-4 MOTUs for *G. insensibilis* (5.0-14.0% K2P). The detected MOTUs did not show any sympatry, and both species consisted of several MOTUs within the Mediterranean Sea, but single MOTUs in the Atlantic or within the Black Sea.

CONCLUSIONS

Our findings confirm a certain level of cryptic diversity in brackish *Gammarus* members, comparable to that found in the freshwater representatives, however much more balanced. Yet, taking into account even the most conservative approach of MOTU delimitation, the number of actual species exceeds at least three times the sum of already described species. These results could be of a vital importance especially in light of the ecological relevance of the studied species and their usefulness as bioindicators.

Do non native macroalgae affect amphipod assemblages?

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ABSTRACT

The invasive macroalgae *Asparagopsis taxiformis* host a significantly different amphipod assemblage than the native species *Halopteris scoparia* (in terms of number of species, abundance and community structure).

KEY WORDS

Amphipoda; invasive macroalgae; benthic ecology.

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In spite of the increasing number of marine alien seaweeds in coastal habitats, the ecological impacts of such introductions have been quantified from only a small fraction of them. Moreover, there are major research gaps, particularly about quantitative community effects on small scale (Thomsen et al., 2017). In this sense, in order to understand the consequences of such disturbances it is necessary to improve our knowledge about how changes in macroalgae composition and structure modify their epifaunal assemblages. Mobile epifauna are major contributors to the flux of materials in rocky habitats, providing most of the total secondary productivity on these environments and acting as a prey source for the majority of demersal fishes (Taylor, 1998, Edgar & Aoki, 1993). Within mobile epifauna, Amphipoda constitute one of the dominant group and because of their abundance, ubiquity, relevance and sensitivity to environmental changes, they are often used as a model group in ecological studies (e.g. Sánchez-Moyano & García-Gómez, 1998; Duffy & Hay, 2000).

The present study aims to explore the effects of the invasive algae *Asparagopsis taxiformis* on am-

phipod assemblages in shallow rocky habitats of Southern Spain. Unlike the closely related species *Asparagopsis armata* (first reported in Spain in 1930) the presence of *A. taxiformis* in Spanish waters is more recent (1999). By now, it is commonly distributed along the coast of South Spain and it has displaced native species (such as *Halopteris scoparia*) as well as previous canopies of *A. armata*, providing arguments to consider it an invasive species (Altamirano et al., 2008).

Samples were collected during July 2014. Five stations (rocky shores separated by hundred of meters) were chosen and three different sampling sites were selected in each station. Within each site, four replicate samples of both the dominant native (*H. scoparia*) and invasive seaweed (*A. taxiformis*) were collected. Samples were preserved in 70% ethanol and later examined in the laboratory using binocular microscopes. The volume of each algae was calculated and all amphipod specimens were counted and identified to species level. Multivariate and univariate analyses were conducted following a multifactorial design with the following factors:

Algae (a fixed factor with two levels: *Asparagopsis* and *Halopteris*), station (a fixed factor, orthogonal with algae and with five levels) and site (Random factor nested with Station and with 3 levels) ($n=4$).

More than 16500 amphipod individuals were found in the whole study, belonging to forty-four different species. Our results highlighted a marked impoverishment of the amphipod community associated with the establishment of *Asparagopsis taxiformis*; the invasive alga showed a significant decrease in both number of species and abundance values (Fig.1). This was a constant pattern, being not affected by factors “Station” or “Site”. Regarding multivariate analyses, the native and invasive species of macroalgae showed different amphipod assemblages (Fig. 2). *Aora spinicornis* and *Stenothoe monoculoides* were dominant in both species of algae. *Gammaropsis maculata*, *Microdeutopus chelifer* or *Hyale* spp. were very abundant on *H. scoparia* and *Caprella acanthifera* was dominant only on *A. armata*. The native algae showed higher

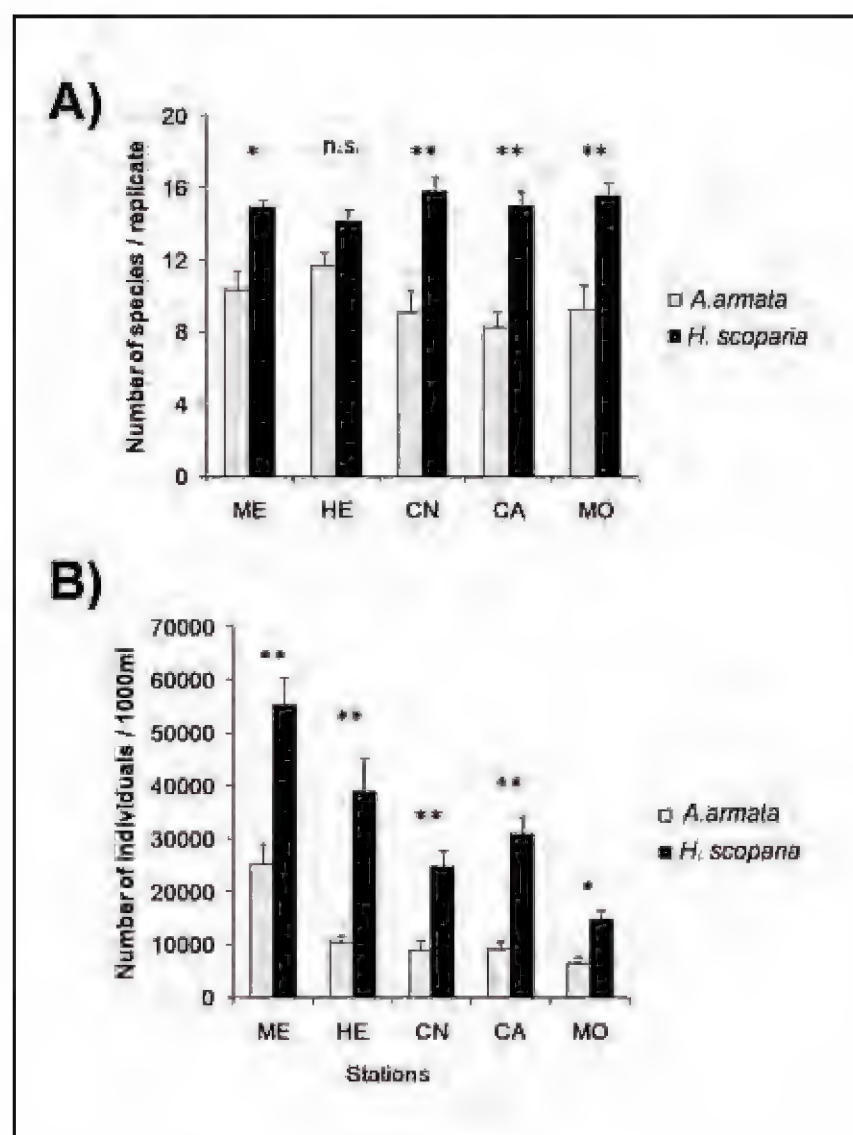


Figure 1. Abundance (B) and Species richness values (A) (Mean \pm SE) found both in *Asparagopsis taxiformis* and *Halopteris scoparia* individuals in each sampling station. ME = Marina del Este; HE = La Herradura; CN = Cantarriján; CA = Cañuelo; MO = Molino de Papel; * = $p<0.05$; ** = $p<0.01$; n.s = non significant.

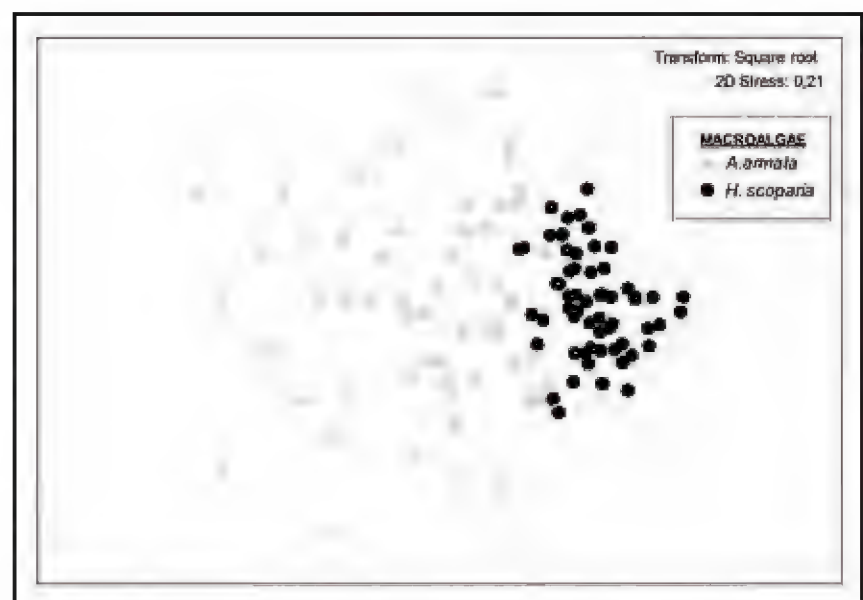


Figure 2. Two-dimensional non-parametric multidimensional scaling (nMDS) plot for amphipod community in both species of macroalgae.

variability among replicates as well, whereas the native species hosted a homogeneous and more constant amphipod assemblage. These results are discussed with previous related studies, as well as with the role of invasive non native macroalgae in the dispersion and assessment of other exotic species.

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Who is the winner? – the demon vs killer shrimp under mesocosm conditions

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ABSTRACT

Dikerogammarus villosus (Sowinsky, 1894) and *D. haemobaphes* (Eichwald, 1841) (Gammaridae) are the most successful invaders in European waters and often co-occur in colonized areas exhibiting similar ecological demands that lead to interspecific competition. We aimed to test in the long term experiment if goby fish may impact the strength and direction of the interaction between the two species.

KEY WORDS

Invasive species; Ponto-Caspian; interspecific interactions; predation.

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INTRODUCTION

Two Ponto-Caspian gammaridean species *Dikerogammarus villosus* (Sowinsky, 1894) (the killer shrimp - Dv) and its close relative *D. haemobaphes* (Eichwald, 1841) (the demon shrimp - Dh) (Gammaridae) belong to the most successful invaders in European large rivers, and in the last decade spread to the inland waters of the U.K. In many rivers, the demon shrimp was the first colonizer, but a drastic diminishing of its population was observed after the introduction of the killer shrimp. Kinzler et al. (2009) did not show any difference in predatory strength between both species thus intraguild predation was excluded as a force leading to the species displacement. However, the active avoidance of the killer shrimp by the demon shrimp was observed by Kobak et al. (2016) leading to the faster spread of Dh in the experimental tank, suggesting

the latter to be a weaker competitor. Both studies were only short-term laboratory experiments.

Aim: The aim of the study was to test if the killer shrimp may negatively influence the population of demon shrimp in a long time experiment and if the presence of a top predator - the racer goby (*Babka gymnotrachelus*) may influence this interaction.

MATERIAL AND METHODS

We conducted a six-month experiment in twelve 700L mesocosms. The experiment consisted of four treatments: 1. only *D. haemobaphes* (Dh); 2. Dh and goby fish; 3. Dh and *D. villosus* (Dv) (introduced one week after Dh); 4. Dh and Dv (introduced one week after Dh) and goby fish. We also conducted additional short term experiments upon the selec-

tive predation of the racer goby on both gammarid species.

RESULTS

In short term experiments, we showed that both gammarids avoided the racer goby predation with a similar success utilizing available substrate. However, when no shelter was available, Dh was preyed more often than Dv. In the mesocosm study, we observed a negative impact of the goby presence on the demon shrimp density, but this effect was independent of the presence of the killer shrimp. We also observed the lower number of Dh in the treatments where Dv was present, regardless of the presence of fish. The occurrence of Dv was independent of the presence of fish, but till the end of the study the density of this species remained much lower than that of Dh.

CONCLUSIONS

Our study showed that the dynamics between two closely related species of invasive gammarids leading to the dominance of the killer shrimp could be explained by a complex of biological features presented by both species. Błońska et al (2015) showed that this species was not a profitable prey for the racer goby possibly due to its hard exoskeleton and, the same, relatively high handling cost. Jermacz et al. (2017) showed that the killer shrimp was attracted, instead of being threatened, by the predator odor. Also in our mesocosm study this species did not avoid the space inhabited by a racer goby. Demon shrimp, even if presenting similar success in utilizing shelters to avoid goby predation, exhibits strong escaping behavior from the stressor, i.e. predator or competitor. This avoidance of the direct interaction with Dv may possibly best explain the low densities of Dh in the presence of Dv observed in nature, as Dh can always find a new area to spread into. Also, we showed that the competition for a shelter did not lead to the higher Dh susceptibility to predation by racer goby, which was

expected to be an important mechanism, as already shown for *Gammarus pulex* (Linnaeus, 1758) (Beggel et al., 2016).

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The status quo of the IceAGE project

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ABSTRACT

Marine amphipods from Iceland are investigated.

KEY WORDS

Iceland; genetic fingerprint; *Lepechinella*; *Rhachotropis*.

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Owing to the complex hydrography of the Icelandic waters, they are of particular interest for studying patterns of diversity changes with depth. Senckenberg am Meer (DZMB) is housing a huge amount of crustacean material collected during two IceAGE (Icelandic marine Animals: Genetics and Ecology) expeditions in 2011 and 2013. The animals are collected around Iceland, between Greenland and Norway from 150 to 3000 m depth. So far 25 000 amphipod individuals are sorted by numerous enthusiastic colleagues to 42 (!) families during two amphipod workshops in Wilhemshaven, Germany and Spala, Poland; the first workshop was entirely funded by the Volkswagen Stiftung. Analyses for two transects (Iceland - Faroes and Norwegian Channel) along biogeographic barriers (submarine mountains and depth gradients) are shown. Special attention is drawn to the genera *Lepechinella* Stebbing, 1908 (Lepech-

inellidae) and *Rhachotropis* S.I. Smith, 1883 (Eusiridae).

A first genetic fingerprint of subarctic amphipods via barcoding was proceeded. The molecular analysis of 270 individuals (belonging to 23 families) from 27 stations resulted in 167 sequences (representing 21 families). Automatic Barcoding Gap Discovery (ABGD) allowed to distinguish 79 Molecular Operational Taxonomic Units (MOTUs). Out of all distinguished MOTUs only five have publicly available sequences (in GenBank and BOLD). What is more, deep structuring was recorded in two taxa, which can express the geographical separation of studied individuals. The COI analysis was supplemented by 16S rRNA gene study.

An outlook for the IceAGE project, including an upcoming expedition, is presented. IceAGE is providing us with a new understanding of the deep-sea benthos in the subarctic.

***Gammarus roeselii* Gervais, 1835 (Gammaridae) in Western and Central Europe: post-glacial colonisation or human mediated introduction?**

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ABSTRACT

The study has shown that in Central and Western Europe *Gammarus roeselii* Gervais, 1835 (Gammaridae) is a postglacial newcomer from the Panonian Basin and Northern Balkans.

KEY WORDS

Post-glacial colonization; population expansion; molecular population structure.

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INTRODUCTION

Gammarus roeselii Gervais, 1835 (Gammaridae) is an epigeal species commonly found in European rivers and, sometimes, in lakes. The species is easy to tell apart from most other local species of *Gammarus* by presence of large dorsal spines on the metasome part of the body. Its range expands from France and Netherlands on the west, to Poland on the east and Greece on the south. The species is known to have diversified during Neogene in the Balkans including many divergent lineages (Grabowski et al., 2017). On the opposite, only one lineage is suspected to have colonised the more northern regions of Europe in historical times.

The aim of the study is to define the geographical origin of *G. roeselii* population from the western and central Europe, if only one lineage is involved and to reveal whether this population fits the model of demographic/spatial expansion. We

also discuss whether the present distribution of this species in the studied area is an effect of spontaneous post-glacial colonisation or anthropogenic factors.

MATERIAL AND METHODS

A 533 bp long portion of the mitochondrial cytochrome oxidase I (COI) marker from 401 specimens collected from 39 locations from Central and Western Europe were amplified. Neighbor-Joining phylogram was produced in MEGA 6 for basic reconstruction of phylogeny. Relationships between haplotypes in populations from the presumably newly colonized part of Europe were illustrated with Minimum Spanning Network (MSN) constructed in POPART v1.7. Mismatch Distribution (MD) analysis with Tajima's D, Fu's F calculated in ARLEQUIN v3.5 were used to assess demographic state in these populations. Demographic

changes in time were plotted using Bayesian Sky-line (BSP) analysis in BEAST v1.8.2.

RESULTS

The populations of *G. roeselii* occurring in western and central Europe derive from only one lineage from the northernmost part of the Balkan Peninsula and from the Pannonian Plain i.e. from the Danube catchment (Black Sea basin). MSN suggested presence of two haplotype groups widely spread in western and central Europe. The obtained star-like topology of MSN for these two groups as well as results of the MD analysis and neutrality tests suggest that western and central European populations of *G. roeselii* are in the state of both demographic and spatial expansion. BSP shows that already in early Holocene, the species has started its colonization up the Danube system. On the other side, its current distribution suggests that the species crossed the main watersheds only

after large navigable canals were built and joined the Black, Mediterranean, North and Baltic Sea basins.

CONCLUSIONS

Most probably, the spread of *G. roeselii* north- and westwards has started as a natural process soon after the Last Glacial Maximum. However, our data suggests that the present distribution of the species results from a combination of natural and anthropogenic factors.

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Preliminary study of heavy metal assessment and their distribution within the hepatopancreas of the talitrid amphipod *Orchestia mediterranea* Costa, 1853

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ABSTRACT

Individuals of the talitrid amphipod *Orchestia mediterranea* Costa, 1853 were collected from the banks of Bizerte lagoon (North of Tunisia) and exposed to a series of contaminated soil according to different concentrations of copper and zinc during three weeks. Specimens were weekly weighed and body metal concentrations of both metals were measured in the talitrid. The bioaccumulation factor was determined revealing that *O. mediterranea* is considered as a macroconcentrator of copper and zinc. This result was assessed by the study of the effect of heavy metals on the hepatopancreas using the Transmission Electron Microscopy.

KEY WORDS

Lagoon; *Orchestia mediterranea*; copper, zinc; bioaccumulation factor.

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INTRODUCTION

In crustaceans, the accumulated concentrations of trace metals vary widely between metals and between taxa (Rainbow, 1998). Many authors such as Phillips & Rainbow (1994) and Rainbow (1995) highlighted that talitrid amphipod are being increasingly used as biomonitors of trace metal bioavailabilities in coastal waters, their accumulated metal concentrations being time integrated measures of their exposure to all sources of bioavailable forms of a metal over a defined time period. This study aimed to assess the effect of copper and zinc on some physiological parameters and to highlight the impact of these metals on the hepatopancreas in the talitrid amphipod *O. mediterranea* Costa, 1853.

MATERIAL AND METHODS

Adult specimens of *O. mediterranea* were collected from the banks of Bizerte lagoon (37°13'8"N/09°55'1"E) and kept in contaminated soils. The heavy metals used were in the form of copper chloride (CuCl₂) and zinc chloride (ZnCl₂) according to Köhler et al. (1996). Two replicates were prepared for each concentration with 10 individuals per replicate. A control was also prepared in which soil was sprayed only with distilled water. Then, specimens were maintained at 20°C under LD 16:8 during 21 days of exposure. Amphipods were weekly weighed. At the end of the treatment, for each metal and for each nominal concentration, an aliquot of both soils and specimens was dried

(24h at 40°C) in order to determine metals concentrations using flame atomic emission spectrometry (FAAS-6800, Shimadzu). The degree of accumulation has been expressed as bioaccumulation factor (BAF). The BAF was calculated by dividing the concentration of the chemical compound in the organism by its concentration in the substrate on which it has been feeding (Mazzei et al., 2014). As proposed by Dallinger (1993), the BAF was used to classify *O. mediterranea* as a macroconcentrator (BAF>2), microconcentrator (1<BAF<2) or deconcentrator (BAF<1). In order to test body mass variations between concentrations and between weeks, Kruskal Wallis and Friedman tests were respectively applied using the Xlstat software.

RESULTS AND DISCUSSION

Results showed that from the first week until the end of the experiment, some fluctuations were observed in the two metals. Concerning the comparison of body mass between nominal concentrations, the Kruskal-Wallis test revealed a highly significant difference whatever the metal. However, Friedman test revealed no significant difference in this parameter between weeks.

According to Hopkin & Martin (1984) the bioaccumulation factor is a useful way to assess heavy metal bioaccumulation capacity. Our results showed that the BAF of the metals used was greater than 2 whatever the nominal metal concentration, revealing that *O. mediterranea* is a macroconcentrator for copper and zinc (Table 1).

The greater metals accumulation may be explained by less effective Cu and Zn excretion mechanisms (Witzel, 1998). Schill & Köhler (2004) suggested the existence of variable species-specific strategies towards metal accumulation and excretion in Crustacea.

In the studied species, the hepatopancreas of untreated animals showed histological and ultrastructural features similar to those described in other crustacean's species (Mazzei et al., 2014). However the different concentrations of Cu and Zn induced significant changes both in the general morphology and in the ultrastructural organization of S and B cells in hepatopancreas. Indeed, the control of *O. mediterranea* store excess Cu and Zn metals granules detoxified in its hepatopancreas. The number of these granules as well as the number of mitochondria and lipid droplets increased with more exposure to metals. Moreover, a destroyed microvillus and a cells bursting were observed. The present findings suggest that *O. mediterranea*, which is abundant in this study site, can usefully be employed in biomonitoring programs.

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Groups	<i>O. mediterranea</i>	Soil	Concentration factor (CF)
Control	131.34±0.00	25.24±2.50	5.6
Zn 300 mg L ⁻¹	125.90±10.06	21.03±1.91	5.99
Zn 400 mg L ⁻¹	137.36±11.35	19.16±2.62	7.28
Zn 500 mg L ⁻¹	153.82±55.30	33.50±1.70	4.56
Control	97.54±0.00	18.85±7.69	4.02
Cu 100 mg L ⁻¹	98.11±14.02	11.03±1.62	9.08
Cu 150 mg L ⁻¹	135±7.61	11.14±1.68	12.31
Cu 200 mg L ⁻¹	176.29±8.21	19.22±6.89	9.72

Table 1: Zn and Cu content (mg.Kg⁻¹, dry weight; means±SD) in soils and in whole body of *O. mediterranea* after exposure for 21 days to various metal concentrations, together with the concentration factor (CF).

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Light pulses effects on the locomotor activity rhythm of *Orchestia montagui* Audouin, 1826 (Amphipoda Talitridae)

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ABSTRACT

The locomotor behaviour of the talitrid *Orchestia montagui* Audouin, 1826 (Amphipoda Talitridae) was studied in the Bizerte lagoon banks in summer under constant darkness with two short light pulses applied around the dusk and the dawn respectively. Results revealed that whatever the experimental conditions, under continuous or interrupted darkness by pulses, two endogenous components have been highlighted. In fact, Periodogram analysis showed the presence of ultradian and circadian periods. The locomotor activity rhythm of this species was better defined under pulses and specimens were significantly more active under continuous darkness. Moreover, a great variability around the activity time was observed especially with pulse of 1h.

KEY WORDS

Coastal lagoon; *Orchestia montagui*; endogenous rhythm; constant darkness; pulses.

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INTRODUCTION

Orchestia montagui Audouin, 1826, a talitrid amphipod species, commonly occurs on sandy and rocky Mediterranean coasts, within decaying sea-grass, beach wrack or gravel

The species is generally associated with slow-drying sediments that are covered with plant debris, cobbles and/or boulders (Lowry & Fanini, 2013). This species, abundant in the banks of Bizerte lagoon, is collected with seven talitrid species (Jelassi et al., 2012). It is characterized by a diurnal locomotor behavior (Jelassi & Nasri-Ammar, 2013). In the present work, we aim to study the effect of light pulses on the locomotor behavior of this species.

MATERIAL AND METHODS

Adult individuals of *O. montagui* were collected

manually in the morning during summer under *Cymodocea banquette*, and between *Suaeda maritima* and *Salicornia arabica* roots, from Bizerte lagoon. Individuals of each species were transported to the laboratory in Perspex boxes. For each experience, 30 individuals transferred, singly to separate annular actograph with humid freshly collected substratum and a small amount of carrot given as food. Then, they were kept in a controlled environment cabinet maintained at a constant temperature of 18 ± 1 °C. Each experience was performed during 13 days. Animals were maintained under constant darkness (DD) in the first five days and under constant darkness with pulses in the rest of experiment. Pulses, of 1 or 2h, were applied at the end of the subjective day as well as at the end of subjective night. The experimental conditions were similar to those described by Bohli-Abderrazak et al. (2012). Data were initially presented for analysis in the form of double-plotted actograms showing activity

accumulated over 20min, using the Chart software package version 35 (School of Biosciences, University of Birmingham, UK). Subsequent periodogram analysis was performed using the program based on the method of Dorscheidt and Beck (Harris & Morgan, 1983). The different locomotor parameters were calculated for each individual. Statistical analyzes were performed using the χ^2 , Wilcoxon and Kruskal-Wallis rank tests.

RESULTS AND DISCUSSION

At the end of the four experiences, 60.8 % of individuals were found alive. The percentage of mortality exceeds 50 % when pulses were applied around dawn. Under constant darkness, results showed the existence of a clear drift to the right explaining the lengthening of the circadian period: the animals started their activity later from day to day. The presence of the drift from the first day showed the absence of photoperiodic memory in *O. montagui* contrary to other amphipod species such as *T. saltator* (Bohli-Abderrazek, 2016) as well as the isopod species *Tylos europaeus* (Bohli-Abderrazak, 2016) that characterized by a good photoperiodic memory. This drift observed under constant darkness was maintained under pulse but less important. Furthermore, the activity profiles are in majority unimodal. The most activity peak showed a slipping of its location from the middle of subjective night under constant darkness to the middle of subjective day under pulse. Whatever the experimental conditions, periodogram analysis highlighted the existence of circadian component slightly shorter under pulses than constant darkness. The shortest circadian period and the most important inter-individual variability ($\tau_{DD+pulse}=24h38'\pm4h34'$) were observed under pulse of 2h around the dusk. The same analysis showed an ultradian period around 12h expect when pulses were applied around the dawn. Globally, the locomotor activity rhythm of *O. montagui* was better defined under pulses and specimens were significantly more active under continuous darkness. Moreover, a great variability around the activity time was observed especially with pulse of 1h. Phase shift varied according to experimental conditions. Moving from continuous darkness to continuous darkness interrupted by pulses, most of them described negative values; therefore, all mean values were

negative describing phase delay. They were equal to -163.6 ± 76.4 (DD, 1 h/dawn), -174.1 ± 53.8 (DD, 1 h/dusk), -169.2 ± 41.3 (DD, 2 h/dawn), and -134.1 ± 78.8 (DD, 2 h/dusk). The Kruskal-Wallis test, revealed no significant difference.

At the end of this study, it would be interesting to multiply the number of points of light intensities and sweep the day as well as the night in order to highlight the phase response curve of the diurnal species *O. montagui*.

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A detailed study of Harpiniinae from two sites on the West African Continental Margin

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ABSTRACT

A clear and definitive understanding of the taxonomy of species, especially in areas where knowledge is limited, such as the deep-sea is needed. Here we report on the use of Environmental Impact Assessment macrofaunal specimens to study the taxonomy and ultimately the biogeography of Harpiniinae (Phoxocephalidae) from West African deep waters. This study indicates that the Harpiniinae are far more speciose than originally reported, and that detailed taxonomic study can contribute to environmental management of poorly studied areas.

KEY WORDS

Harpiniinae; Phoxocephalidae; deep-sea; amphipoda; taxonomy; Angola Basin.

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INTRODUCTION

Global climate change and human derived habitat destruction continues to impact biodiversity across the globe (Cardinale et al., 2012). In light of these threats, biological monitoring is essential to understand, and subsequently preserve, species diversity (Glover & Smith, 2003). Central to this aim is a clear and definitive understanding of the taxonomy of species (Kim & Byrne, 2003), especially in areas where knowledge is lacking, such as the deep-sea (Worm et al., 2006).

The deep-waters of the West African Continental Margin region have a rapidly expanding deep-water oil and gas industry, and these commercial activities legally require Environmental Impact Assessment surveys (EIAs) to be completed (Kuedikuenda & Xavier, 2009). EIAs generate a substantial amount of macrofaunal material and environmental data, which can be used for further research. Amphipods are a typical component of ba-

thyal (1000–4000 m) macrofaunal assemblages (Barnard & Drummond, 1978).

Amphipod samples at these depths in the Angola Basin are dominated by Phoxocephalidae, particularly the sub-family Harpiniinae. The group consists of 10 genera: *Harpinia*, *Harpiniopsis*, *Pseudharpinia*, *Basuto*, *Cocoharpinia*, *Coxophoxus*, *Feriharpinia*, *Heterophoxus*, *Proharpinia* and *Torridoharpinia* (Barnard & Drummond, 1978), with a total of 134 species. Harpiniinae, like all peracarids, brood their young, thus restricting distributions and accelerating cryptic speciation, making them notoriously difficult to identify. New Harpiniinae species are regularly discovered in deep waters; however due to the problems in identification, crucial work in revising species, genera and determining their phylogenetic relationships have not yet been undertaken (Jarrett & Bousfield, 1994). It is currently difficult to clarify how much of the morphological variation is due to ontogenetic variation or sexual dimorphism and what the species specific

characters are. By studying the detailed morphology and subsequent scoring of characters of the Harpiniinae (Phoxocephalidae), we have highlighted that the specimens are far more speciose than originally reported. Much of the deep-water macrofauna from the West African region is likely to be new to science, and identification keys for the region currently do not exist. This study aims to improve the knowledge of the Harpiniinae and to revise character delineation within the group.

STUDY AREA

The Angolan Basin (~5°S to 25°S and 5°W to 8°E) off the Namibian and Angolan coast north of the Walvis Ridge reaches a depth of about 5500 m. The samples from this study come from two baseline surveys undertaken in the northern region of the Angolan Basin in 2000 and 2008 respectively. The two sites were geographically parallel, ~150 km apart and the depths in the offshore region were ~1000 m deeper. Site 1, shelf adjacent, (6° 25S, 012° 10E) depths ranging from 1200 to 1500 m. Site 2, offshore, (6° 50S, 010° 50E) depths ranging from 1400 to 2454 m. The main feature in the region is the Congo River, supplying fauna in the deep-sea with large amounts of particulate organic matter (Sibuet & Vangriesheim, 2009).

RESULTS AND CONCLUSIONS

Following preliminary analysis of 45 individual core samples from site 1 and 86 from site 2 it was noted that diversity (species richness) increased with depth, and that this covaried with decreasing sedimentary organic matter, indicating competition for resources. Additionally, differences were apparent in the number and composition of Harpiniinae morpho-types between the two sites. The macrofauna community from site 1 comprised of 3778 individuals, of 137 morpho-types. Although the amphipods only represent 2% (89 ind.) of the whole community assemblage, 50% of all amphipods were Harpiniinae (37 ind.). Site 2 had 4603 individuals from 292 morpho-types (> twice the number of species as study area 1). Here the amphipods represented 7% (321 ind.) of the community assemblage, however Harpiniinae only represented 30% of all amphipods, 3% of total fauna (89 ind.). The fauna of site 1, was originally reported as comprising 10 amphipod morpho-

types, from 6 families, including three morpho-types for Phoxocephalidae (A-C). However, re-identification and detailed study has revealed 20 morpho-types from 6 families, although the family composition has changed. The three morpho-types of Phoxocephalidae have now increased to 13 distinct morpho-types, 11 of which were from the sub family Harpiniinae. The fauna of site 2 revealed 6 additional morpho-types than were previously identified, including specimens from the genus *Pseudharpinia*. This study has highlighted that the Harpiniinae within the samples are far more speciose than originally reported, and indicated that there are distinct differences between the two study areas. An important finding for future environmental management of the region.

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New records of alien Amphipoda in Mediterranean marinas

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ABSTRACT

In this study we present new data regarding the distribution of non-indigenous amphipods in both marinas and recreational vessels across the Mediterranean Sea. We observed six non-indigenous amphipod species, two of which are firstly reported for the Mediterranean Sea. The caprellid *Caprella scaura* Templeton, 1836 occurred in most of the inspected marinas.

KEY WORDS

Non-indigenous species; recreational boating; Mediterranean Sea.

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INTRODUCTION

The continuous movement of yachts and sailing boats across the Mediterranean Sea is suspected to contribute to the introduction and spread of marine non-indigenous species. In order to analyse the phenomenon, we investigated fouling communities from both marinas and recreational vessels across the Mediterranean Sea. We focused on amphipods because they are very successful colonizers of artificial hard substrates, reaching high densities in bio-fouling communities. Moreover, they lack pelagic planktonic larval stages and they have little autonomous dispersal capability, meaning that their occurrence in non-native regions can confidently be attributed to human transport.

MATERIAL AND METHODS

We selected 30 marinas across the northern rim of the Mediterranean Sea, according to their relevance as tourist hotspots, maritime connections and vessel traffic: Alicante, Barcelona (Spain); Antibes, Cannes, Port Camargue, Cap D’Agde, Grand

Motte, St. Tropez, St. Maxime, Cogolin (France); Rome, Sorrento, Ischia, Palermo, Ragusa, Licata, Siracusa, Marzememi, Riposto (Italy); Grand Harbour and Msida (Malta); Heraklion, Agios Nikolaos, Rhodes (Greece); Karpaz, Famagusta (Cyprus); Fethiye, Bodrum, Marmaris, Finike (Turkey). In each marina, we collected samples from the docks, other submerged structures and from vessels hulls. Samples were collected from April to November 2015 and 2016. Amphipods were separated from the other benthic organisms and identified to the species level. Non-indigenous status was assigned to species satisfying Chapman & Carlton (1991) criteria.

RESULTS AND DISCUSSION

In our samples we found the following NIS: *Ampithoe bizseli* Özaydinli et Coleman, 2012. It was first described from samples collected on the West coast of Turkey and on the East coast of Africa. This species may have been introduced into the Mediterranean by travelling on ship hulls from the Indian Ocean to one of the big harbours of Tur-

key. In our study, individuals of *A. bizseli* were found in hull fouling of boats in Cyprus, namely in Karpaz and Famagusta marinas.

Aoroides longimerus Ren et Zheng, 1996. The genus *Aoroides* was known to be exclusively distributed in the coastal regions of the Pacific Ocean (Ariyama, 2004). Then, in 2015, it was found in the French Atlantic coast (Gouillieux et al., 2015), in the Bay of Brest (NW Brittany) and associated to oyster reefs in Arcachon Bay (SW Brittany). In our research, we found aorids matching the characters of *A. longimerus* in boat samples from Port Camargue.

Bemlos leptochirus Walker, 1909. It is an Indo-Pacific species which was previously recorded in the Mediterranean Sea only once on the coasts of Egypt (Bellan-Santini & Ruffo, 1998), and thus it was characterized as a likely lessepsian migrant. We found individuals from this species in Crete (Greece), namely in Heraklion and Agios Nikolaos marinas.

Caprella scaura Templeton, 1836. It was first described from individuals collected in Mauritius, but the real origin of the species is actually unknown. Further records suggested that its native range may include Brazil, Caribbean Sea, Australia and Japan (Ramalhosa & Canning-Clode, 2015). It was recorded for the first time in the Mediterranean Sea in 1989 (Scipione, 2015), in Taranto (Italy). Since then, it has been reported in several Mediterranean countries, proving to be one of the most widespread non-indigenous species of Europe (Galil et al., 2014). It was present in most of our sampled marinas, and 90% of the boats carrying caprellid amphipods included *C. scaura*.

Erichthonius pugnax Dana, 1852. It is a species native to the Indo-Pacific region. A species with striking morphological similarity was found in the Lagoon of Venice and originally described by Krapp-Schickel (2013) as *Erichthonius dydimos*. In our study, we found individuals of *E. pugnax/dydimos* in the hull fouling of boats in Port Camargue, Cap d'Agde (France). Given that these findings occurred nearby two hotspot sites of introduction of Japanese species, Venice Lagoon and Thau Lagoon, we hypothesize a NW-Pacific origin for the *Erichthonius* material.

Stenothoe georgiana Bynum & Fox, 1977. Its geographical native range extends from Chesapeake Bay (Virginia) to Tampa Bay (Brazil), in-

cluding North Carolina and Florida. In 2010, it was found for the first time in the Mediterranean Sea, in off-coast fish farms of Alicante and Murcia (Spain) (Fernández-González & Sanchez-Jerez, 2017). During our investigations, we found individuals of *S. georgiana* in marinas and hull fouling of boats in France (Port Camargue, Cap d'Agde, Saint Tropez), Italy (Sicily and Sorrento) and Malta (La Valletta).

CONCLUSIONS

This study demonstrates that non-indigenous species are frequently occurring in Mediterranean marinas, and on the hulls of boats travelling in the Mediterranean Sea. Only a few marinas did not exhibit any alien amphipod. Analysis of some taxonomically challenging families is still ongoing, therefore the final number of non-indigenous amphipods may prove higher than the one here presented.

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Friend or enemy? Chemical recognition and reciprocal responses among native and alien amphipods

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ABSTRACT

In aquatic environment, intra- and interspecific communication is often associated with infochemicals used for detection of mates, predators and food. The ability to respond adequately to environmental cues is crucial for invasive species, facing unknown communities. On the other hand, the ability of aliens from the same native region to recognize one another is likely to increase their fitness. In our experiment we analysed the ability for chemical recognition among invasive freshwater Ponto-Caspian gammarids (*Dikerogammarus villosus*, *Dikerogammarus haemobaphes* and *Pontogammarus robustoides*) and native for Europe *Gammarus fossarum*.

KEY WORDS

Gammarids; biological invasions; communication; kairomones.

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INTRODUCTION

Mutual recognition, detection of food supply and ability to recognize threats in aquatic ecosystems are strongly connected with chemical stimuli (Ferrari et al., 2010). Before any invasive species is successfully established in a new territory, it has to face a huge variety of scents which are unknown and have never been experienced in their native region.

They form new interactions with local biota and can re-create reciprocal links among organisms originating from the same region if any are present (Strauss et al., 2006). Nevertheless, the adaptation to a new territory is connected with gaining the capabilities of evoking behaviour adequate to chemical stimuli.

MATERIAL AND METHODS

We studied intra- and interspecific chemical recognition among invasive freshwater Ponto-Caspian gammarids (*Dikerogammarus villosus*, *Dikerogammarus haemobaphes* and *Pontogammarus robustoides*) and a non-invasive European *Gammarus fossarum*. We used a y-maze with constant inflow of water (0.5 L min⁻¹) to the y-arms from two separate source tanks containing the signal (the scent of living animals or the alarm cues released by crushed individuals) or control water, respectively. We introduced 10 gammarids into the mixing zone of the y-maze. Each experimental trial lasted for 35 min, including 5 min for adaptation and 30 min of behavioural observations. The experiment was replicated 10 times for each signal type

and species. The behaviour of animals was recorded and afterwards analysed with Noldus Ethovision® XT 10 software. We analysed three variables: (1) time spent in the y-maze zones, (2) number of entrances to the y-arms and (3) activity.

RESULTS

Only *D. villosus* was attracted to conspecific and heterospecific alarm cues as well as to living heterospecifics, but not to living conspecifics. Other Ponto-Caspian species avoided the scent of *D. villosus*, whereas *G. fossarum* decreased its activity in its presence. We conclude that *D. villosus*, as the strongest competitor and intra-guild predator (including cannibalism) among the tested species, perceived various scents as potential food cues and posed a threat to other gammarids.

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Soft bottom amphipod (Gammaridea and Senticaudata) communities of Vasiliko bay (Cyprus, East Mediterranean Sea)

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ABSTRACT Diversity and composition of amphipod taxo-composition of Vasiliko bay, Cyprus.

KEY WORDS Mediterranean Sea; Cyprus; Gammaridea; Senticaudata.

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The island of Cyprus is located in the Levantine Sea, known for its ultra-oligotrophic waters, high species diversity but low species abundance. Current knowledge on soft-bottom benthic macrofauna community composition in south coasts of the island is considered limited. In summer 2013, a first attempt to collect background information on this matter was initiated, as part of a research project, targeting to Vasiliko bay and near-by coastal area which is influenced by multiple human induced stressors. As part of this study, the Amphipoda (Gammaridea, Senticaudata) assemblages are investigated in regards to environmental gradients.

Three (3) replicated benthic macrofauna samples were collected with Van-Veen grab (surface of 0.1 m²) from 42 sampling sites, interspersed in study area. Samples were sieved with a 0.5 mm

mesh, fixed in formalin and stained with Rose Bengal. At MER's laboratory, benthic macrofaunal organisms were sorted and identified to lowest taxonomic level possible. Within Crustacea, amphipods dominated. The Amphipoda identification was carried out at the laboratory of IMBBC, HCMR Crete by following the latest updated taxonomic keys. A check list of species was composed along with their biogeographical distribution. A series of multivariate analysis (e.g. nMDS, ANOSIM, SIMPER) were performed to identify relationships among amphipod assemblages (in terms of abundance and diversity), environmental gradients (depth, sediment type and organic matter) and presence of human stressors. The results of the study will be presented and discussed at the conference.

A Phylogeny and Classification of the Amphipoda with the establishment of the new order Ingolfiellida (Crustacea Peracarida)

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ABSTRACT

Order Ingolfiellida stat. nov., four new suborders: Pseudingolfiellidea stat. nov.; Colomastigidea stat. nov.; Hyperiopsidea stat. nov.; and Amphilochidea stat. nov. are here presented.

KEY WORDS

Amphipoda; Order; Suborder.

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The current higher classification of the Amphipoda is not phylogenetic and is consequently much in need of revision. Our study developed a new classification for the order Amphipoda using a morphologically based phylogeny. The analysis was based on a DELTA database to the families of world amphipods. Heuristic searches of 115 characters were performed using PAUP. Our analysis employed an iterative process between DELTA, PAUP and MacClade, and the choice of starting tree was unimportant as long as it was a parsimoniously shortest tree. When a number of shortest trees were available they were not significantly different from each other. A most parsimonious tree was analysed in MacClade to determine which characters were potentially useful synapomorphies, which characters were uninformative and which characters appeared to be homoplastic. Further analyses were then performed until the shortest tree with the most resolved synapomorphies resulted. In the analyses presented, almost 100 iterations were performed. Statistical measures for node support were not used because such meas-

ures do not test the quality of synapomorphies and in addition are effectively phenetic. Molecular studies often question some details of classifications previously based on morphology, but molecular studies utilise extremely few taxa and as a consequence they never propose novel molecular-based classifications to replace existing morphological-based classifications. Our study included 223 families. Molecular methods do not give consistent results across studies and often produce results that are inexplicable in the light of pre-existing knowledge. Molecular methods do not replace morphological methods, but they should be viewed as complimentary. We compare our methodology with previous methodologies and give examples where morphological and molecular results coincide.

Our analysis resulted in one new order Ingolfiellida stat. nov., four new suborders: Pseudingolfiellidea stat. nov.; Colomastigidea stat. nov.; Hyperiopsidea stat. nov.; and Amphilochidea stat. nov. which join the existing suborders Hyperiidea and Senticaudata. Within the six suborders there are

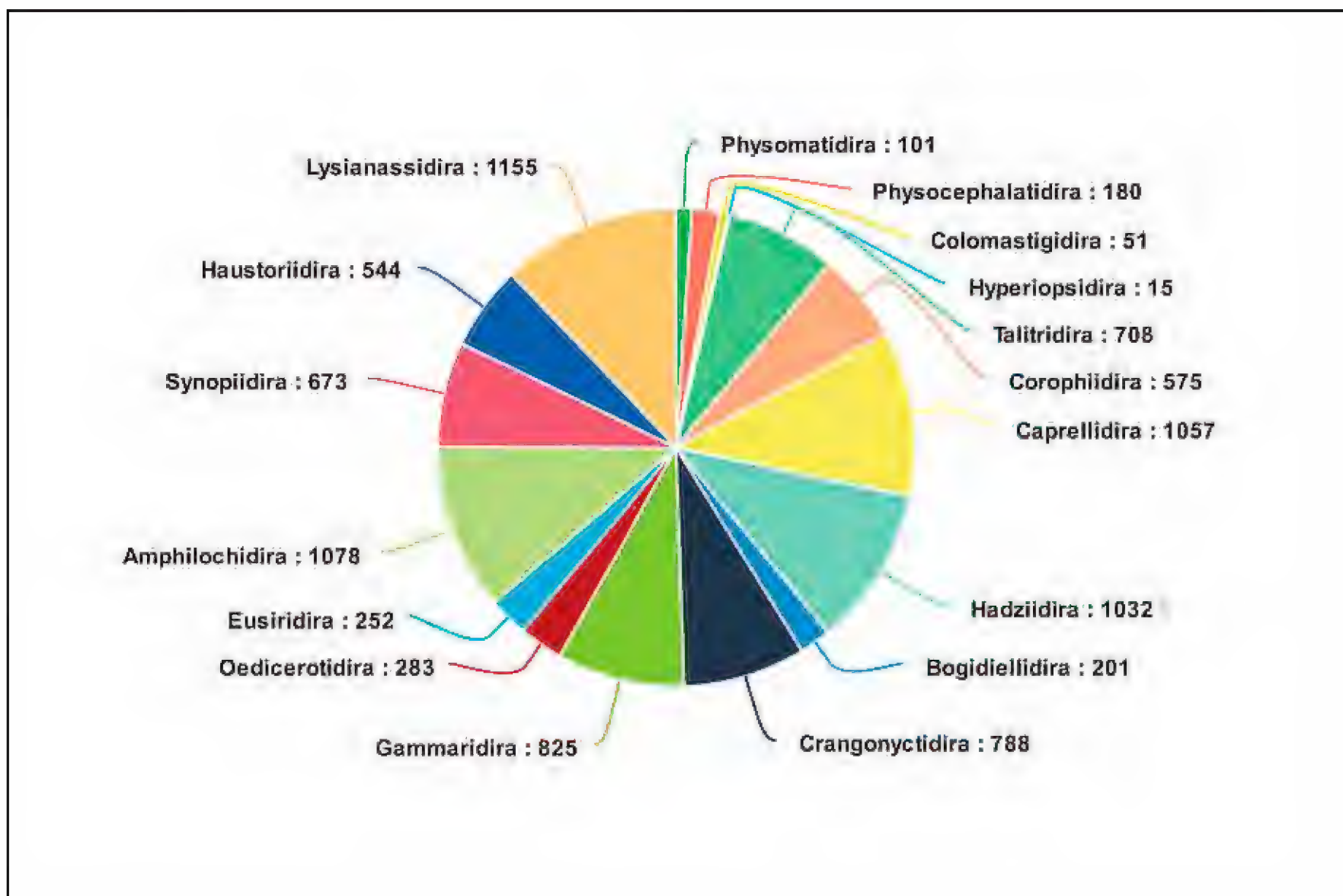


Figure 1. The relative species contribution of each parvorder to the Amphipoda. Excluded from pie chart: Carangoliopsidira (2 species); Maxillipiidira (3 species); Pagetinidira (4 species); Podosiridira (1 species); Pseudingolfiellidira (4 species).

13 infraorders, 5 of which are new and 23 parvorders, 15 of which are new.

In the parvorder analysis we chose *Metaingolfiella* Ruffo, 1969 as the outgroup. The Ingolfiellida are the sister group to the Amphipoda and *Metaingolfiella* lies at the base of the ingolfiellidan clade (Ruffo (1969) and Vonk & Schram (2003). For the analyses of the Amphilochidira, Physomatidira and Physocephalatidira we chose *Pseudingolfiella* Noodt, 1965 as our outgroup because it was shown in our parvorder analysis to be at the base of the amphipod clade. In our analysis of one infraorder (Lysianassida) we used Lundberg rooting. This gave us much better resolution on the tree than did *Pseudingolfiella*.

Cladistic trees, are presented illustrating the re-

lationships of the Amphipoda at parvorder level, the families of the Physomatidira and Physocephalatidira, the families of the Maxillipiidira, Oedicerotidira, Eusiridira and Amphilochidira and the families of the Synopiidira, Haustoriidira and Lysianassidira. These trees are discussed.

Of the parvorders (Fig. 1), the Lysianassidira includes the greatest number of species (1155) followed by the Amphilochidira with 1078, the Caprellidira with 1057, the Hadziidira with 1032 and the freshwater parvorders Gammaridira and Crangonyctidira which together contain 1613 species - about 62% of all amphipod species. The remaining parvorders are relatively small, none with more than about 700 species, but together they contribute 48% of species.

Locomotor activity rhythms in talitrids from Mediterranean coastal ecosystems. Part II: Italian case

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ABSTRACT

Jointly presented, the two sets of experiments (Italian and Tunisian) on circadian rhythms in talitrids (*Talitrus saltator*, *Orchestia gammarellus*, *Orchestia montagui*), give a broad view on the observable variation in talitrid behaviour. The same species, observed throughout the Mediterranean, may exhibit common and different traits depending on the season, environment, impacts and latitude.

KEY WORDS

Circadian rhythms; Mediterranean coastal wetlands.

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INTRODUCTION

Talitrids have been studied for rhythmic behaviour (Bregazzi & Naylor, 1972; Williams, 1983). The question rises if all the species or the populations of the same species react in the same way to the cues that define the cyclic environment. We recorded locomotor activity rhythm of specimens collected along Italian coastal ecosystems with the same techniques and protocol as for Tunisian populations (Nasri-Ammar et al., 2017). The challenging aim of this comparison was the reinforcement of the knowledge about talitrid locomotor activity and/or the hint of a further source of variability in endogenous rhythms.

MATERIAL AND METHODS

Talitrus saltator was collected on i) sandy beaches with and without vegetated dunes (Maremma Regional Park, Grosseto) and on ii) a nourished beach at the S. Rossore Regional Park (Pisa); *Orchestia*

gammarellus was collected on iii) a dune slack, iv) a river bank, v) the entrance of a cave (Maremma Regional Park); *Orchestia montagui* was collected in vi) a seagrass banquette (Talamone Port, Grosseto). We hand collected adult individuals and recorded their activity at the Department of Biology in Florence starting the same day of collection. Period (in hours) and rhythm definition (Signal-to-Noise Ratio, SNR) were calculated on 21 days for each individual (Rossano et al., 2008).

RESULTS AND CONCLUSIONS

A study performed to define the effect on rhythms of species ecology was carried out at the Maremma Regional Park, on *T. saltator* from a sandy beach with and without dune, *O. montagui* from a *Posidonia* banquette and *O. gammarellus* from the entrance of a cave and river bank (Rossano et al., 2008). The circadian rhythm was more clearly expressed by *T. saltator* and *O. montagui* (period around 24h and high definition), in line with the

results obtained in Tunisia with the Korba population. *O. gammarellus*, on the other hand, had a variable period and low definition, and results were comparable to those obtained at the Bizerte Lagoon in Tunisia, while the same species did not show the same features at the Ghar El Melh Lagoon (Tunisia).

The seasonality of the rhythm in *T. saltator* was demonstrated by the longer and less precise pattern of activity of samples collected in winter compared to summer (Nardi et al., 2003) on two sandy beaches in Tuscany, Castiglione della Pescaia (Grosseto) and S. Rossore Regional Park. The results are the opposite of the results obtained at Korba Beach in Tunisia, where a clearer period was observed in winter, probably due to latitude effect and extreme temperatures reached in summer.

Two sympatric species were compared, *T. saltator* and *O. gammarellus*, colonizing a sandy beach and dune slack at the south end of Collelungo Beach at the Maremma Regional Park. A clear difference between species in the expression of the rhythmic activity was actually observed in summer, may be due to the colonization of different areas of the beach (*T. saltator* burrows in the moist sand and *O. gammarellus* occupies the rush *Juncus acutus* bushes).

At S. Rossore Regional Park, where nourishment activities were implemented along the sandy beach, *T. saltator* were collected at two sites a few kilometers apart. Talitrids expressed a clear period with small variability in autumn at the most impacted site, at odds with the expectation of a poorer rhythm expression.

A set of recordings on three sub-populations previously identified by Ketmaier et al. (2009) was performed at Collelungo Beach, which specifically showed a dependence of the expression of rhythm on the erosion gradient (from river mouth toward the furthest sites). The results showed an unexpected decrease in rhythm precision in summer across the erosion gradient.

The comparison among Italian and Tunisian

populations highlighted common features of species responses when the environmental features are comparable and unravelled a further scale of variation that can be observed when the latitude affects the local environmental conditions.

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The scent of danger - direct and indirect effects of predator presence on mesograzing amphipods

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ABSTRACT

The authors present a study that tested for the combined effects of interspecific competition and predator avoidance in *Echinogammarus marinus* (Leach, 1815) (Gammaridae).

KEY WORDS

Apparent competition; trait-mediated interactions; habitat segregation; *Gammarus*.

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Predation has direct impact on prey populations by reducing prey abundance. Additionally, predator presence alone can also have non-consumptive effects on prey species, potentially influencing their interspecific interactions and thus the structure of entire assemblages. The performance of potential prey species may therefore depend on both the presence of predators and competitors. We studied habitat use and food consumption of a marine mesograzing crustacean, the amphipod *Echinogammarus marinus* (Leach, 1815) (Gammaridae), in the presence/absence of a fish mesopredator, the sea scorpion *Taurulus bubalis* (Euphrasen, 1786) and/or an amphipod competitor, *Gammarus locusta* (Linnaeus, 1758). The presence of the predator affected both habitat choice and food consumption of the amphipods, indicating a trade-off between the use

of predator-free space and food acquisition. Without the predator, individuals were equally distributed over different microhabitats, but in the presence of the predator, most animals hid under a provided shelter and reduced their food consumption. Additionally, habitat choice of the amphipods changed in the presence of the competitor, also resulting in reduced feeding rates. The behaviour of *E. marinus* is apparently driven by trait-mediated direct and indirect effects caused by the interplay of predator avoidance and competition. This highlights the strong non-consumptive impacts of mesopredators on amphipod prey organisms. The flexible responses of small crustacean consumers to the combined effects of predation and competition may substantially sculpt the structure of coastal ecosystems and the multiple species interactions therein.

“Where did you come from, where did you go?” - ‘Cosmopolitan’ amphipod species in the age of global ship traffic and blue growth

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ABSTRACT

The authors present a study designed to detect the origin of select cosmopolitan amphipod species. It is assumed that their recent worldwide distribution is the result of ancient invasive events prior to the rise of scientific documentation.

KEY WORDS

Biological invasion; *Jassa marmorata*; *J. slatteryi*; *Monocorophium acherusicum*.

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Several amphipod species have repeatedly been reported from different locations all over the world. Some of these reports were assigned retrospectively to misidentifications and/or to the description of endemic and formerly cryptic species. A few of these cosmopolitan species, however, are confirmed to occur in geographically separated locations around the world. Since the beginning of scientific documentation, species such as *Monocorophium acherusicum* (Costa, 1853), *Jassa marmorata* Holmes, 1905 and *J. slatteryi* Conlan, 1990 inhabit fouling communities on artificial substrates such as harbours and all kinds of offshore installations in warm-temperate marine regions. We hypothesize

that the recent distribution of these cosmopolitan fouling species is the result of several invasive events, mediated by transoceanic shipping activities probably initiated centuries ago. Here we present the first preliminary results of a study, designed to determine the former native ranges of these species. Specimens from different locations around the world were analysed both morphologically and using genetic tools for studying the phylogeographic patterns based on the variation of the barcoding gene COI. Possible pathways, age and number of introductions are discussed and set in context with historic reports and former assumptions for the occurrence of the three species.

Molecular phylogeny and biogeography of freshwater amphipods in Italy: state of the art

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ABSTRACT

More than one hundred amphipod species are known so far in Italian freshwaters; however species delineation remains uncertain and evolutionary history elusive. Using a morphotaxonomic approach combined with DNA-based delimitation methods, we demonstrated that traditional species are polyphyletic, and that cryptic species richness is high both in surface and ground waters. The Amphipod historical biogeography of Italy is discussed in the light of these discoveries.

KEY WORDS

Morphotaxonomy; molecular taxonomy; species delimitation; historical biogeography.

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Following Fauna Europaea and related databases (de Jong et al., 2015), the Italian freshwater amphipod fauna includes 17 genera attributed to 11 families. Ninety-four species are listed in these data banks, besides approximately ten species with taxonomic status to be clarified. Three genera - the epigeal *Echinogammarus* and *Gammarus*, and the predominantly subterranean *Niphargus* - make up for over 60% of all Italian species. Only two surface, non-invasive, alien species are known. The biodiversity patterns outlined by morphospecies analysis reveal higher species richness in the northern areas and a percentage of endemic species greater than 60% (Ruffo & Stoch, 2006). Unfortunately, despite previous detailed morphological studies, species delineation remains quite uncertain in most genera (particularly *Echinogammarus*, *Rhipidogammarus*, *Niphargus*, and *Salentinella*), mainly due to parallel and convergent evolution and, especially for subterranean species, to paedomorphic heterochronic events (Stoch & Galassi, 2010). Moreover, a proper understanding of amphi-

pod biogeographic and evolutionary history remains elusive. To address this problem, we have been using a morphotaxonomic approach combined with DNA-based delimitation focusing on the contribution of amphipod phylogeography in the areas of biogeographic history (and the relative roles of dispersal and vicariance), colonization history, and cryptic species diversity patterns. We also aimed to test for differences in surface and groundwater species richness: considering that subsurface environments are more fragmented than surface ones, we hypothesized that cryptic species richness should be higher in ground waters.

Samples of freshwater amphipods have been collected in over 1,000 locations in Italy and neighboring countries (southern France and Corsica, Malta, Austria, Dalmatia, as well as Greece and Crete island: Fig. 1) and preserved in 96% ethanol. After DNA extraction using a commercially available kit (Qiagen Dneasy Blood & Tissue), independent nuclear (28S) and mitochondrial (COI) markers were amplified and sequenced for at least

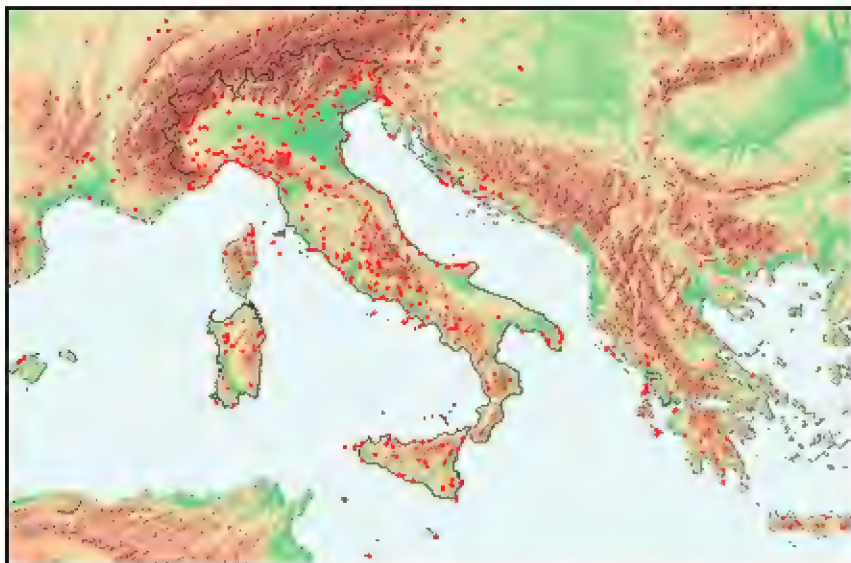


Figure 1. Sampling sites where freshwater amphipod specimens were collected for molecular analysis.

one individual per site. Topotypic specimens of almost all named species and subspecies, including junior synonyms, were collected.

Preliminary results cast a new light on the diversity and biogeography of Italian amphipods. In particular, traditional species groups based on morphology turn out to be polyphyletic, both in surface and ground waters; cryptic species richness (affecting up to 100% of known morpho-species in some families using a COI divergence threshold and/or Poisson tree processes models: Eme et al., 2017) is high both in surface (especially in the *Echinogammarus*-genera complex) and in ground waters (*Niphargus*, *Salentinella*). These results falsify our hypothesis that cryptic species richness is higher in subterranean waters. Moreover, our results suggest that, while the number of species present in Italy should be more than doubled compared to what was previously known (i.e. 203 species identified so far), the number of genera should instead be reduced from 17 to 15, because of oversplitting based on morphology at this higher taxonomic level. Molecular results do not modify the species richness patterns delineated using morphospecies analysis, with species richness decreasing with decreasing latitude. Both surface and groundwater clades turn out to be important in defining the historical biogeography of the Italian peninsula and its main islands. Paleo-Thyrrhenian (originating from the fragmentation and movement of the Corso-Sardinian and other microplates), trans-Adriatic and some trans-Ionian clades (some of them reaching the Thyrrhenian coast as well as Sardinia, maybe due to dispersal

during the Messinian salinity crisis: Stoch et al., 2016) form the core of the paleo-endemics that have shaped the Italian fauna, together with some older relics (*Metacrangonyx*, *Pseudoniphargus*) that present distribution patterns probably deriving from the fragmentation of the Tethys Sea (Bauzà-Ribot et al., 2012). More recent invaders of Balkanic origin are found in the Pre-Alpine regions, while affinities with southern France and regions north of the Alps are limited to a few cases, demonstrating the important role of the Alpine chain as a barrier to amphipod dispersal. Finally, the small part of the Dinaric Karst included in the north-eastern part of Italy presents a few merodinaric elements (sensu Sket, 1984) that add further complexity to the biogeographic puzzle.

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Amphipods from the arctic hydrothermal vent field “Loki’s Castle”, Norwegian Sea

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ABSTRACT 11 species of amphipods are found at the arctic hydrothermal vent Loki’s Castle, Norwegian Sea, belonging to 5 families. 2 species are new to science.

KEY WORDS Hydrothermal vents; amphipod variety.

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INTRODUCTION

The hydrothermal vent field “Loki’s Castle” located in the north Norwegian Sea at 73°N at 2350 m depth is a series of black smokers on an ultra slow-spreading ridge. Since its discovery in 2008, the area has been sampled yearly by the University of Bergen, now providing a thorough representation of the fauna connected to the system. The area consists of a 30 m high mound of hydrothermal deposits with two main clusters of chimneys reaching up to 13m and a sedimentary area with more diffuse venting and smaller barite chimneys. The vent-fluids peak at approximately 320 °C at the main fluid exits, but cool rapidly to 30–40 °C at the base of the chimneys and in the barite field. The prevailing temperature in the surrounding area is slightly negative (-1 °C).

RESULTS AND CONCLUSIONS

The amphipods represented in the collected ma-

terial count 11 species, representing the gammaridean families Calliopidae, Melitidae, Oedicerotidae, Sebidae as well as the hyperiidean family Hyperiidae. So far, two new species have been described from the material, one from the new genus *Exitomelita* (Melitidae) and one *Monoculodes* (Oedicerotidae). Calliopidae and Oedicerotidae are most abundant in number specimens as well as number of species. Calliopids have been registered at hydrothermal vents in the Pacific, whereas Oedicerotids seem to be represented at the most examined hydrothermal vents in both the Atlantic and Pacific oceans. Curiously, the Lysianassoids and Pardaliscids, present as scavengers at Pacific hydrothermal fields, have not been found at Lokis Castle, neither have the Eusirids of the more southern Atlantic vent fields.

We see a clear difference between amphipods living on the chimneys (*Exitomelita sigynae* Tandberg, Rapp, Schander, Vader, Sweetman et Berge, 2011), those close to the vents (mainly Oedicerotids) and those in the more diffuse venting areas and distant barite fields (all families represented).

Exitomelita sigynae is the only amphipod species where ectosymbiont bacteria have been registered.

The presentation will give an overview of the

species represented in this isolated system and discuss their trophic interactions. Possible biogeographic connections with other areas of hydrothermal vents will be discussed.

Diversity of the subterranean genus *Niphargus* Schiødte, 1849 (Amphipoda Niphargidae) in Spain

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ABSTRACT Present knowledge of the subterranean genus *Niphargus* Schiødte, 1849 (Amphipoda Niphargidae) in Spain is given.

KEY WORDS Subterranean waters; *Niphargus*; taxonomy; Spain.

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Fauna of the subterranean genus *Niphargus* Schiødte, 1849 (Amphipoda, fam. Niphargidae) in Spain is only partially known, and nearly 8 species of this genus is mentioned for Spain.

Recently some new taxa of this genus were described: *Niphargus notenboomius* G. Karaman, 2015 (loc. typ.: Angles, 16 km WSW of Gerona, Spain) and *Niphargus laisi geronensis* G. Karaman, 2015 (loc. typ.: 2 km from Garriguella

Spain). Very large variability and limited number of localities of known species makes difficult recognition of some taxa, and further study on new samples from various new localities is necessary to establish real taxonomical status of some *Niphargus* taxa in Spain. Some known taxa, mentioned many years ago from Spain, *N. puteanus* (Koch, 1836), *N. longicaudatus* (A. Costa, 1851), etc., needs their reexamination and confirmation.

A first state of the art on the ecology and biology of the amphipod genus *Themisto* Guérin, 1825, a key pelagic predator in temperate and polar oceanic regions

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ABSTRACT

A state of the art of the knowledge on of the amphipod genus *Themisto* Guérin, 1825, a key pelagic predator in temperate and polar oceanic regions, is presented together with new results on their genetic connectivity, ecology and feeding habits.

KEY WORDS

Amphipoda; Hyperiidea; ecology; pelagic; polar; evolution.

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Of the major mesozooplankton predators in high-latitude oceans, pelagic amphipods are amongst the least studied groups. Hyperiid amphipods represent a group of exclusively pelagic species of which many co-evolved with soft-bodied zooplankton groups such as salps and hydromedusae, using them as substrate, for food, shelter or reproduction.

Amphipods of the hyperiid genus *Themisto* Guérin, 1825, currently comprising seven distinct species, have so far been neglected in ecological and molecular studies despite their role as key players in temperate and cold-water pelagic ecosystems. In some areas they represent the base of entire food webs and the major prey for many commercially important fish and squid stocks. *Themisto* amphipods are major predators of meso- and macrozooplankton in several regions from shelves to open ocean and the poles to the subtropics.

In the northern high latitudes there are indications that range shifts are occurring and this is also expected in the warming waters of the Southern Ocean, highlighting the urgent need to gather the current state of knowledge and identify the many knowledge gaps. This will allow us to reliably evaluate, monitor and predict the consequences of potential distributional and ecological shifts.

We discuss distributional patterns, life history traits, feeding habits and role in regional food webs and biogeochemical cycles of *Themisto* amphipods and point out some key questions and hypotheses on their ecology and biology that so far remain unanswered or untested.

Moreover, we will present the first molecular results on *Themisto* species from the polar oceans, and discuss their genetic structure and historical demography in the light of their evolutionary ecology.

Finally, we will complement this with some behavioural observations and experimental data on feeding experiments carried out on *Themisto* species from both hemispheres.

Amphipod-pteropod associations in the Antarctic Polar Frontal zone and the Weddell Sea

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ABSTRACT

Reports on associations between hyperiid amphipods and aducted pteropods are presented as anti-predatory behaviour in the Southern Ocean.

KEY WORDS

Hyperiididea; Southern Ocean; pelagial; predator deterrence.

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Hyperiid amphipod crustaceans are abundant in Southern Ocean waters from the Polar Frontal Zone to shelf waters where they represent a major prey for fish and seabirds. *Hyperiella dilatata* Stebbing, 1888 has evolved a peculiar anti-predatory behaviour: it abducts chemically-protected, shell-less pteropods from the water column and carries them on their dorsal side, holding it between its pteropods (McClintock & Janssen, 1990). The pteropod *Clione antarctica* E.A. Smith, 1902 produces de novo a predator-deterrent chemical named pteroenone (Bryan et al., 1995). Tandem pairs of pteropods and amphipods were significantly less predated upon than single individuals of *Hyperiella* Bovalius, 1887 and hence this behaviour is very efficient as protection from visually hunting icefish in the crystal-clear shelf waters around the continent

(McClintock & Janssen, 1990), however, in the open ocean this behaviour was so far undocumented. We report this association in open waters sites in the Southern Ocean and hypothesize against which potential predators this anti-predatory behaviour may have been selected for.

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Global phylogeography of *Gammarus lacustris* G.O. Sars, 1864, the most widespread freshwater amphipod species

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ABSTRACT

A phylogeographic analysis of *Gammarus lacustris* G.O. Sars, 1864 across its Northern Holarctic range reconfirms a subdivision into two main lineages, which meet in Eastern Europe.

KEY WORDS

Gammarus lacustris; phylogeography; systematics.

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Gammarus lacustris G.O. Sars, 1863 (Gammaridae) is the most widespread of all freshwater amphipods, with a range extending from northwestern Europe eastwards across the Holarctic, up to northeastern North America. It mostly inhabits mountain lakes in the central parts of the continents but also lowland waters towards the north. Much of this range must have been colonized post-glacially. Several other Central European *Gammarus* taxa have recently been split into complexes of local, deeply diverged cryptic species, but inferences from studies of *G. lacustris* have been confounding. Adding two new broad datasets from across Northern Europe and North America to the previously available mtDNA COI sequence data, we reconfirm a basic subdivision of *G. lacustris* into two major lineages, which correspond to the W and E allozyme races of Vainio & Väinölä (2003). One is distributed

in Central and North-West Europe (e.g. the Alps, the British Isles, and Scandinavia up to northern Norway: this is the typical *lacustris* clade, encompassing the “*G. alpinus*” of Alther et al. (2016), which needs to be synonymised). The other one reaches from NE Fennoscandia and Eastern Europe through Eurasia and the entire North American distribution, including the range of the putative *G. limnaeus* Smith, 1874 in NE North America (the *limnaeus* clade). The sequence divergence between the clades is 6 %, which is less than differences among regional cryptic species of other gammarids. The ranges of the main clades also overlap, and mtDNA alone might not suffice for determining their taxonomic rank. There is further phylogeographic structuring within each clade (differences of 1–2%), informative of wide dispersal from a number of Pleistocene refugia.

Locomotor activity rhythms in talitrids from Mediterranean coastal ecosystems. Part I Tunisian case

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ABSTRACT

A comparative study was conducted on activity rhythms of some talitrid species from Mediterranean coastal wetlands, particularly sandy beaches and coastal lagoon banks. Sympatric species and populations of the same species from different coasts were compared across Tunisian and Italian coasts. The same recording methods were used under constant conditions to highlight the main characteristics of the endogenous rhythms of the selected species and their variation. We present here a general introduction of the subject and the Tunisian case.

KEY WORDS

Circadian rhythms; *Talitrus saltator*; *Orchestia gammarellus*; *Orchestia montagui*.

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INTRODUCTION

Locomotor circadian rhythmicity is an adaptation necessary to cope with periodic environmental changes and to anticipate extreme conditions. In the case of talitrids low air humidity and high temperatures must be avoided for survival. Therefore the microenvironment may determine the expression of rhythmic behaviour. Among adaptive strategies, burrowing into the sand in the eulittoral and supralittoral zones and sheltering within vegetation or stranded debris are expressed by sandhoppers and beachhoppers, respectively. It was interesting to understand the variability of circadian rhythm characteristics in different populations, considering the diversity of Mediterranean coastal ecosystems, both in the northern and southern coasts. In Tunisia wetlands are subject to high fluctuations in water regimen and temperatures, with a typical seasonality of arid and hot summers and wet winters. Water inputs

are generally temporary. We compared the expression of the endogenous activity rhythms between populations within the same species and between species within the same season and between seasons.

MATERIAL AND METHODS

Adult specimens of *Talitrus saltator* (Montagu, 1808) were collected under *Posidonia oceanica* banquette from Korba (with dune) and Berkoukech (without dune) beaches. During summer Korba beach is more used for leisure tourism than Berkoukech beach. *Orchestia montagui* Audouin, 1826 and *O. gammarellus* (Pallas, 1766) species were collected, under *Cymodocea* banquette and between *Suaeda maritima* and *Salicornia arabica* roots, on Bizerte and Ghar El Melh lagoon banks. Individuals of both species were transported, in Perspex boxes, to the

laboratory of the Department of Biology, University of Tunis and immediately transferred, singly, into recording chambers with humid freshly collected substratum and a small amount of carrot given as food. To highlight the endogenous locomotor rhythm, the experimental specimens were kept under constant darkness in a controlled environment cabinet maintained at a constant temperature of 18 °C ± 1 °C. Raw data were displayed as double-plot actograms, showing cumulative activity values per 20 min intervals, (software Chart35; School of Biosciences, University of Birmingham, UK). These data were then subjected to periodogram analysis (program TIME SERIES software package).

The percentage of animals showing periodicity, mean circadian period and Signal-to-Noise Ratio (SNR) were calculated for each individual from the raw data.

RESULTS

The activity rhythm phenotypes showed a significant variation and the inspection of the actograms indicated that the circadian and ultradian (when it existed) rhythmicity may vary, both within and between species. Globally, the percentage of periodic animals was significantly lower in *Talitrus saltator* from Korba beach. The free-running period changed within individual recording sessions. *Orchestia montagui* individuals, in which

endogenous activity followed a significant circadian period, were typically diurnal unlike the other species that were nocturnal. The circadian periods of *T. saltator* from the two beaches were approximated closely to 24 h, whereas those of *O. montagui* and *O. gammarellus* were longer as may be seen in Table 1. The free-running activity rhythms were approximately in phase with the subjective diel cycle for the first days, then they drifted progressively, as the free-running period increased. The definition of the rhythm also varied between individual records (Table 1). To obtain more objective estimates for species comparison, signal-to-noise ratio values derived from the periodograms of individuals were used. The mean SNR values corresponding to the circadian component were higher in *O. montagui*, *O. gammarellus* and *T. saltator*, respectively from Bizerte lagoon, Ghar El Melh lagoon and Berkoukech beach.

Moreover results highlighted a seasonal variation in the characteristics of *T. saltator* locomotor rhythm in Korba beach with better defined endogenous circadian rhythms in winter and more unstable ones in summer.

CONCLUSIONS

Our results showed the existence of a circadian and an ultradian components in the locomotor rhythm of *O. montagui*, *O. gammarellus* and *T.*

Sites	Species	Ultradian rhythmicity (%)	Circadian rhythmicity (%)	$\tau_{12} \pm SD$	$\tau_{24} \pm SD$	$Snr_{12} \pm SD$	$Snr_{24} \pm SD$
Bizerte lagoon	<i>Orchestia montagui</i>	10	90	12h53'±0h23'	25h'40±0h37'	0.048±0.006	0.687±0.293
	<i>Orchestia gammarellus</i>	13.3	56.7	12h40'±0h28'	25h29'±1h02'	0.159±0.165	0.279±0.156
Ghar El Melh lagoon	<i>Orchestia gammarellus</i>	36.7	76.7	12h50'±0h41'	25h13'±1h03'	0.222±0.117	0.560±0.302
Berkoukech beach	<i>Talitrus saltator</i>	30	83.33	12h18'±0h7'	24h26'±0h42'	0.092±0.036	0.491±0.290
Korba beach	<i>Talitrus saltator</i>	-	40	-	24h±0h22'	-	0.210±0.160

Table 1. Locomotor rhythm characteristics of *Orchestia montagui*, *Orchestia gammarellus* and *Talitrus saltator* in different Tunisian wetlands (sites). τ_{12} and τ_{24} correspond to circatidal and circadian periods; SNR: signal-to-noise ratio; SD standard deviation.

saltator from Bizerte lagoon banks, Ghar El Melh lagoon banks and Berkoukech beach, respectively. *Talitrus saltator* individuals from Korba beach were characterized by exhibiting only a circadian component almost equal to 24h. The presence of an ultradian component may have an adaptive signification as suggested by Morgan (1991). In fact, these species occupy a dynamic ecological niche, burrowing into the moist sand close to the shoreline and an endogenous awareness of the tide state would enable the amphipods to avoid being stranded above high water on the beach or in the banks of the lagoon. Depending on the type of wetland lagoon or beach and, as in this case, to the human use of the beach and other biotic factors such as the interspecific competition (Jelassi et al., 2014; Jelassi et al., 2015) the observed flexibility of the expression of rhythmic behaviour may represent an important adaptive mechanism through which individuals can increase their fitness in a variable environment.

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Spontaneous activity and orientation behaviour in *Deshayesorchestia deshayesii* (Audouin, 1826) (Talitridae) at Kalaat Landalos Beach, N-E Tunisia

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ABSTRACT

Spontaneous activity and solar and lunar orientation of *Deshayesorchestia deshayesii* (Audouin, 1826) (Talitridae) were observed for 24 hours under natural conditions. The activity began around sunset and finished at sunrise, with two peaks: the first one indicating a landward migration and a second one a seaward migration. Orientation was significantly dependent on spontaneous activity, air humidity and temperature, azimuth and sex.

KEY WORDS

Spontaneous activity; Orientation; Talitridae; Tunisia.

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INTRODUCTION

Activity pattern and orientation are important to cope with changing environmental conditions. Talitrids dwelling sandy beaches are model species to understand how these adaptations are expressed (Pardi & Papi, 1953; Papi & Pardi, 1953; Scapini et al., 2013). Few studies have analysed the relation of spontaneous activity and orientation under natural conditions of *Talitrus saltator* (Montagu, 1808) and *Britorchestia brito* (Stebbing, 1891) in Tunisia and *T. saltator* and *Orchestia gammarellus* (Pallas, 1766) in Italy (Scapini et al., 2002; Colombini et al., 2013). In this study, we analysed the behaviour of *Deshayesorchestia deshayesii* (Audouin, 1826) (Talitridae) on Kalaat Landalos Beach, Ariana, Tunisia.

MATERIAL AND METHODS

A study on the spontaneous activity and solar and lunar orientation of *D. deshayesii* was carried out the 12-13/03/2017 from 9:00 to 9:00 (24 hours). The spontaneous activity on the beach was analysed using cross pit-fall traps that captured spontaneously walking animals from the four directions: seawards to South-East (135°), landwards to North-West (315°), longshore to North-East (45°) and South-West (225°). Pit-fall traps with wet sand at the bottom were placed across the beach on three levels from waterline. Individuals captured in the traps were taken out each two hours and released in the experimental arena immediately thereafter for the orientation tests. The arena permitted the view of the sky, sun or moon (the experiments were car-

ried out one day after the full moon, 99% of illuminated fraction) and permitted landscape view or screened it off, alternatively (Ugolini et al., 1986). Orientation was analysed through the statistics of circular distributions (Batschelet, 1981). Spherically Projected Linear Models (SPLM) were developed from the dataset, using the orientation angles as response variable and environmental and intrinsic factors as predictor variables (Scapini et al., 2002). The best model was chosen with the Akaike criterion and Likelihood Ratio Test (LRT) was used.

RESULTS

Regarding spontaneous activity, a total of 7,424 individuals were captured: 3,485 juveniles, 2,332 females and 1,688 males. Both adults and juveniles started their activity around sunset, at low tide (the tides ranged between a minimum of -0.5m to a maximum of 1.00m).

The entire population ended its activity at sunrise, when tide started to rise. Two peaks of activity were recorded for both juveniles and adults: a first peak was recorded around 23:00, indicating a significant migration towards land whereas the second peak was recorded at 5:00, indicating a massive and significant return towards the shoreline. The orientation tendency of this spontaneous migration was confirmed by the circular analysis of the captures in the cross traps (V test, corrected for grouping).

The orientation landwards had a maximum of precision at 21:00. The distributions of orientation experiments in the arena (carried out in parallel with the study of the spontaneous activity), with or without landscape view, were significantly directed seawards in 23 cases (V test) and only the distribution obtained in the experiment carried out at 1:00 with landscape view was completely dispersed. The best model developed with SPLM analysis was:

Angle \sim spontaneous activity*** \times (humidity*** + temperature***) + azimuth*** + sex

A highly significant ($p < 0.001$, LRT test) dependence resulted of the orientation with spontaneous activity (abundance), air temperature and humidity (in interaction with spontaneous activity) and solar azimuth. Sex was retained by the model, but was not significant; other factors, particularly landscape vision and atmospheric pressure were not influencing.

CONCLUSIONS

This study shows for the first time a nocturnal migration of *D. deshayesii*, which was oriented landwards after sunset and seawards at sunrise. The orientation capability was expressed both at night and during the day, and was independent on the landscape visibility: the sun and moon are therefore the best candidates as orientation cues for this population, as the orientation was seawards throughout day and night, compensating for the azimuthal changes. The orientation variation was dependent on air temperature and humidity, with higher concentration with high temperature and low humidity, thus showing the importance of orientation for survival.

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Abundance patterns of vagile macrofauna associated with the bryozoan *Bugula neritina* (Linnaeus, 1758) in marinas along the Iberian Peninsula. Dominance of crustacean amphipods

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ABSTRACT

The epifauna associated with the bryozoan *Bugula neritina* (Linnaeus, 1758) were studied in 38 marinas along the Iberian Peninsula in 2011 with the aim of describing its faunal composition on a regional spatial scale. Amphipods showed a clear dominance reaching the 86% of the total abundance, followed by tanaids (4%), mollusks (4%), polychaetes (3%) and isopods (2%). Furthermore, additional studies in a temporal scale carried out in 6 marinas in the Cádiz Bay, showed that amphipods were also the dominant group, both in winter and summer season.

KEY WORDS

Amphipods; epifauna; *Bugula neritina*; marinas; abundance; Iberian Peninsula.

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INTRODUCTION

The increasing number of marinas in recent years involves a high amount of artificial structures in marine environments, which could affect the existing ecosystems (Small & Nicholls, 2003; Bulleri & Chapman, 2010). The communities of organisms inhabiting these structures are generally different from those found in adjacent natural habitats (Vaselli et al., 2008). However, the artificial structures effects on the small epibiont invertebrates that inhabit these sessile organisms (or basibionts) have been poorly studied (Guerra-García et al., 2015). Unlike in natural habitats, epibiont fauna associated with artificial structures, especially those that take part of marinas, has scarcely characterized, espe-

cially along large spatial gradients. Therefore, the main objective of the present study was to characterize the composition of the epifauna associated with the bryozoan *Bugula neritina* (Linnaeus, 1758) in several marinas in the Iberian Peninsula and quantify the relevance of amphipods in the global community. This bryozoan was chosen as basibiont for its successfully ability to colonize the surface of those new artificial habitats, specifically in marinas, (Ryland et al., 2011) and for being found in all the studied stations.

MATERIAL AND METHODS

The study area encompassed the Atlantic and

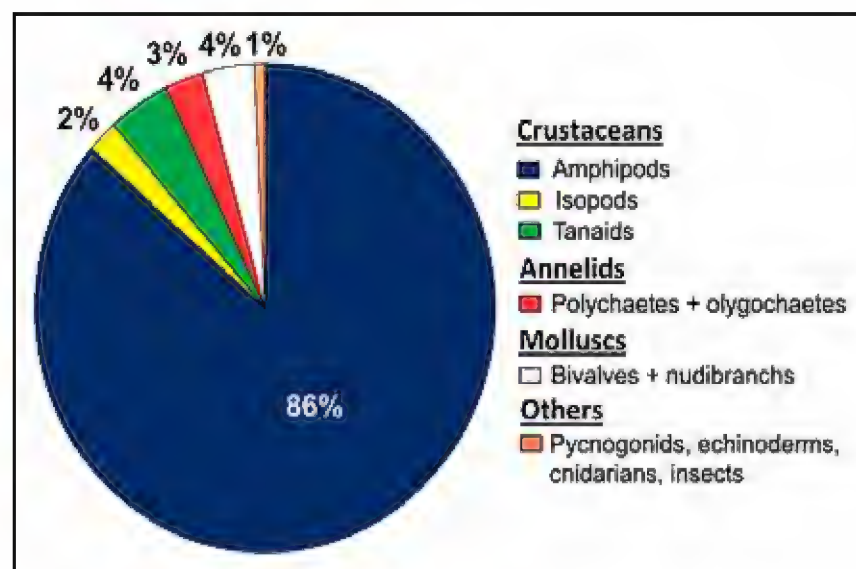


Figure 1. Total abundance percentage of epifaunal groups detected in *B. neritina* samples.

Mediterranean coasts of the Iberian Peninsula. For the spatial scale study, a total of 38 marinas were sampled in late spring and beginning of summer of 2011. Three colonies of *B. neritina* were collected from submerged structures in each marina, close to the water surface and directly removed by hand. Just after collection, the samples were preserved in 90% ethanol (Ros et al., 2015). At the laboratory, the whole macrofauna were separated using a sieve with a mesh size of 0.5 mm and were identified at the level of main groups. The abundance of different faunistic groups were expressed as number of individuals / 1000 ml of substrate (Guerra-García et al., 2015). Additionally, 6 marinas of Bahía de Cádiz were sampled in summer and winter along 2 years (2016 and 2017) using a similar methodology to explore if the dominance of amphipods was consistent in different seasons.

RESULTS AND CONCLUSIONS

Regarding the composition of the fauna within the *B. neritina* samples, different faunistic groups were found: amphipods, isopods, annelids, tanaids, bivalves, nudibranchs, pycnogonids, echinoderms, cnidarians and insects. Amphipods crustaceans were the dominant group in terms of abundance, being the only group present in all marinas and reaching the 86% of total abundance (Fig. 1).

In addition, amphipods showed average abundances ranging from 10000 to 50000 ind./1000 ml

of substrate. This abundance was much higher than the obtained in other studies carried out in natural intertidal and shallow subtidal habitats (Guerra-García et al., 2011). The seasonal study conducted in marinas of Bahía de Cádiz also showed that amphipods were the dominant group, both in winter and summer time. Therefore, construction of artificial structures in the marine environment could be affecting the abundance patterns of epibiont fauna, contributing to an increase in the abundance of some amphipod species. This study highlights the importance in terms of abundances of amphipod crustaceans in artificial habitats and supports its potential role as a key group for monitoring this type of marine environments.

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Genetic variation of the groundwater amphipod *Crangonyx islandicus* Svavarsson et Kristjánsson, 2006, endemic to Iceland

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ABSTRACT

An endemic groundwater amphipod species, *Crangonyx islandicus* Svavarsson et Kristjánsson, 2006 (Crangonyctidae), was discovered in 1998 in the volcanic zone of Iceland. Genetic analyses have revealed different geographic patterns in variation of mtDNA and the nuclear internal transcribed spacer (ITS) region. The mtDNA represents five distinct monophyletic mtDNA lineages within Iceland which divergence follows the geographic distances between the sample sites, supporting an existence of subglacial refugia. A mtDNA lineage from northeast Iceland diverged from the other lineages 4.8 Myrs ago, including samples from north Iceland. Patterns in the ITS marker suggest however that the oldest split is between the north and the south Iceland. ddRadSeq analysis was conducted to analyze this discrepancy, a majority of the nuclear markers support the split observed with the ITS region, indicating admixture of the populations in north Iceland.

KEY WORDS

mtDNA; ddRadSeq; divergence; introgression; refugia; Pleistocene.

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INTRODUCTION

Crangonyx islandicus Svavarsson et Kristjánsson, 2006 (Crangonyctidae), is a groundwater amphipod species, endemic to Iceland, inhabiting lava fields throughout its volcanic zone (Kornobis et al., 2010). It is the only metazoan species known to have survived in subglacial refugia during the Pleistocene, probably in fissures along the tectonic plate boundary in Iceland (Kornobis et al., 2010). A phylogeographic study based on two mitochondrial genes, 16S and COI, revealed a clear divergence of 5 mitochondrial monophyletic clades (Fig. 1a) following the geographical distances within Iceland

and present two putative cryptic species, clade F in northeast Iceland, which diverged about 4.8 Myrs ago from the other clades (Kornobis et al., 2010). Variation at the internal transcribed spacer region (ITS) 1 and 2, showed a different phylogeographic pattern, the ITS scenario (Fig. 1b), potentially driven both by concerted and divergent evolution, showing a main split between samples from northern Iceland and the samples from south Iceland (Kornobis & Pálsson, 2011).

To evaluate the discrepancy obtained with the two markers we used a double digest restriction-site associated DNA sequencing (ddRADSeq) of individuals sampled at four locations belonging to three

of the mitochondrial clades (one in south and two in the north). We evaluated the level of incongruence among loci, and tested the amount of admixture between sampling locations caused by migration or incomplete lineage sorting (ILS).

MATERIAL AND METHODS

Sixty individuals of *C. islandicus* were sampled used dip nets after applying electricity with electric fishing gear, in four springs, representing three distinct mitochondrial clades: AA', E and F (Fig. 1a). Specimens were stored in 96% ethanol and DNA extracted were using a standard phenol chloroform protocol. ddRADseq library was constructed using a modified protocols from Peterson et al. (2012). STACKS v1.3 (Catchen et al., 2011) was used to demultiplex the paired-ends reads of one HiSeq run and two MiSeq runs and assembled de novo into loci using pyRAD v1.3.1 (Eaton, 2014). Sequence variation was analyzed 1) using one SNP per locus and 2) based on haplotypes of all the SNPs present in a locus. After removing loci with Minimum Allele Frequency (MAF) below 5% and loci detected under selection using Bayescan v2.01 (Foll & Gaggiotti, 2008), the partition among sampling sites was summarized with modern Fst-statistics using mmod (Winter, 2012) in R. To take into account the

base call uncertainty, caused by the low coverage dataset, we computed a Fst-like statistic (Fumagalli et al., 2013) based on genotype likelihood implemented in ANGSD using realSFS tools (Kornelissen et al., 2014). Assignments of individual genotypes to different populations were done with DAPC (Jombart et al., 2010) and based on Bayes factor (Leaché et al., 2014). The ABBA-BABA test (Durand et al., 2011) was used to test for introgression (admixture) and ancestral hybridization among the 4 samples.

RESULTS AND DISCUSSION

Seven out of 335 variable loci, presenting in total 3539 SNPs were detected to be under selection, including the histone H3 gene and two mitochondrial regions (NADH subunit 5 and ND2+tRNA-Trp), identified by nblast to genebank. Large majority of the genomic markers (92%) and the differentiation metrics supported the ITS scenario (Fig. 1b), both when analysed with Fst/Phist-like statistics and when based on assignments of individual genotypes and using the Bayes factor. The mtDNA patterns are restricted to a proportion of markers which may be affected by selection or which have survived ancestral admixture of nuclear markers between the two lineages in northern Ice-

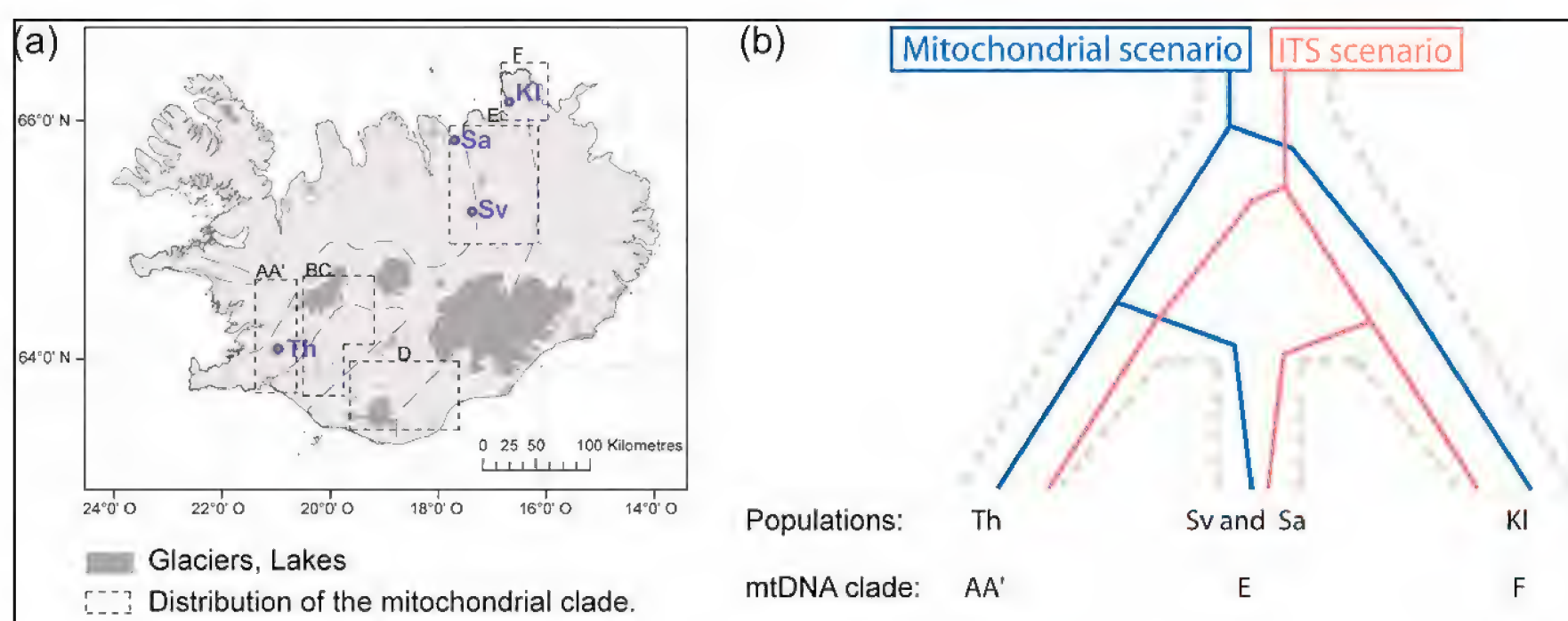


Figure 1. Sampling sites and summary of previous genetic analysis based on mtDNA and ITS genetic variation, (a) Map of the sampling sites (in purple) and schematic distribution, denoted with squares and capital letters, of the mitochondrial clades previously defined by Kornobis et al. (2010). The volcanic zone is marked with curved dotted lines. (b) The two alternatives scenarios of the population relationships proposed in the literature showing a mito-nuclear discordance. The grey dotted line represent the undefined "species tree". Th: Lake Thingvallavatn, Sa: Sandur Adaldal, Sv: lake Svartárvatn, Kl: Klapparos.

land, however the results of the ABBA-BABA test was inconclusive and supported also admixture between the south and the E population in the north.

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Microorganisms associated with the groundwater amphipod *Crangonyx islandicus* Svavarsson et Kristjánsson, 2006

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ABSTRACT

Two endemic subterranean freshwater amphipod species were discovered 1998 and 2000 in Iceland, and *Crymostygious thingvallensis* Kristjánsson et Svavarsson, 2004, and *Crangonyx islandicus* Svavarsson et Kristjánsson, 2006. Little is known of this groundwater ecosystem and nothing is known about its other species or the food source of the amphipods. To explore this system further we studied the micro-biotic community in the groundwater, the hyporheic zone and in the amphipods by targeting 16S RNA for bacteria and 18S RNA for ciliates using the Illumina MiSeq high throughput sequencing technique. Samples were obtained throughout the distribution range of *C. islandicus*. Distinct species composition of bacteria and ciliates was found in the amphipod samples. The results from the groundwater samples gives a good starting point for future research of this isolated groundwater ecosystem within the volcanic zone of Iceland, which appears to be based on chemoautotrophic bacteria.

KEY WORDS

eDNA; refugia; bacteria; ciliates.

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INTRODUCTION

Two endemic subterranean freshwater amphipod species were discovered in 1998 and 2000 from springs in lavafields in Iceland, and *Crymostygus thingvallensis* Kristjánsson et Svavarsson, 2004 (Crymostygidae), and *Crangonyx islandicus* Svavarsson et Kristjánsson, 2006 (Crangonyctidae). These species are found in the groundwater springs in lava fields within the volcanic active zone. Genetic patterns within *C. islandicus* show different monophyletic groups and even cryptic species in different geographic areas which indicates that the group has survived repeated glaciations in Iceland (Kornobis et al., 2010). The findings of such complex organisms as amphipods points to an exis-

tence of an ecosystem in the groundwater of Iceland. The aim of this study is to explore the microbial diversity in the groundwater habitat of *C. islandicus*.

MATERIAL AND METHODS

Crangonyx islandicus was collected from several locations in Iceland. Glass beads (25 gr) were laid out in the source of the springs for eight weeks, and 5 liters of water was sampled from the source of the spring and filtered through Sterivex filters (0.22 µm). DNA was extracted from whole specimens of the amphipods, from the sterivex filters and the glass beads. Bacteria 16S RNA and ciliate 18S

RNA was amplified from the samples and sequenced with MiSeq Illumina. Commands from OBITools were used to process the sequence reads, SILVAngs used to get taxonomic information and R was used for statistical analysis.

RESULTS AND DISCUSSION

Distinct species composition of bacteria (Fig. 1), and ciliates was found in the amphipod samples and in the water sampled. The most abundant bacteria taxa in the amphipod samples were *Shewanella* sp. and *Halomonas* sp. which are groups that have been associated with chemolithotrophy and weathering of basalt (Lysnes et al., 2004; Homann et al., 2009). We suggest that these groups of bacteria might serve as a potential food source for the amphipods or they might be a symbiotic associate, facilitating the living of the amphipods in this food scarce environment as known e.g. for the chemosynthetic bacteria taxa *Thiothrix* in *Niphargus ictus* (Dattagupta et al., 2009). Most abundant ciliate taxa were from

the subclasses Apostomatia and Scuticociliatia. Apostomatia living as epibiont ciliate on *Crangonyx islandicus* has been documented with morphology and Sanger sequencing (Gudmundsdottir et al., 2017). The ciliate genus *Tetrahymena*, was found in equal frequencies from all sample types, amphipods, glass beads and in water samples, indicating that this is a common ciliate in these freshwater springs.

ACKNOWLEDGEMENTS

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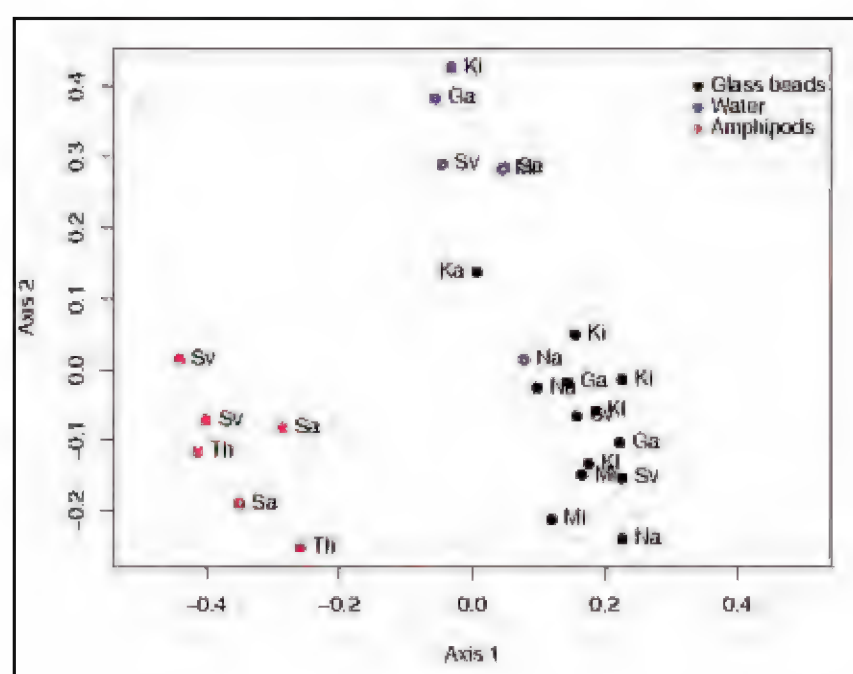


Figure 1. Ordination of species composition of bacteria in amphipods, water samples and glass beads.

A preliminary investigation on diversity and distributional pattern of Amphipods (Peracarida Crustacea) in the intertidal habitats of the South Andaman Islands, India

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ABSTRACT

This study mainly focused on the investigating the amphipod distribution and diversity along the South Andaman Coast as Andaman & Nicobar Island is one of mega bio hotspot in India, but no fruitful study is done till date on this aspect. Distribution and diversity of macrobenthos and their relationships Physico-chemical parameters of the water and sediment in different inter tidal habitats of South Andaman Island, India during different seasons of the year (September 2013-August 2014) at ten locations have been studied. 1442 individuals representing 24 taxa under thirteen families of amphipods were identified. Amplesicidae, Eusiroidea, Urothidae, and Dexaminidae were the most dominant families. Shannon-Weiner ($H' \log_2$) test is done for calculating the spatial diversity and species richness (S) test is done. *Ampelisca scabripes* clearly dominated and frequently occurring the entire region along with *Paracalliopiella* sp., *Paramoera* sp., and *Urothoe* sp.1. Correlation between species and physico-chemical parameters have been calculated.

KEY WORDS

Amphipod; Intertidal; Diversity; South Andaman.

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Amphipods (Peracarida, Crustacea) represent one of the most diverse taxa. Many amphipod species show a high grade of ecological niche specificity and tolerance, which makes them suitable to study the distribution and spatio-temporal changes in environmental conditions. Sedimentary habitats characteristics play a determining role in their community structure and abundance. The primary purpose of this study was to document the distributional pattern and diversity of amphipod population in the intertidal region of the South Andaman coast. Present study deals with the macrobenthic samplings carried out for one year (September 2013-August 2014) at ten locations (Fig. 1) along the

South Andaman coastline. Three replicate samples were collected at each location covering high, mid and low water lines. Altogether 1442 individuals representing 24 taxa under thirteen families of amphipods were recorded. The Amplesicidae, Eusiroidea, Urothidae, and Dexaminidae were the most dominant groups. Overall, Shannon-Weiner diversity index ($H' \log_2$ 2.2 ± 0.92) and species richness (S 6.17 ± 3.6) were recorded highest at Chidiyatapu followed by Havelock Island and Hutbay. Marina Park showed highest numerical abundance (473) followed by Chidiyatapu and Wandoor, whereas Carbyn's Cove showed the lowest abundance. While, evenness is highest at Burmanallah (J:

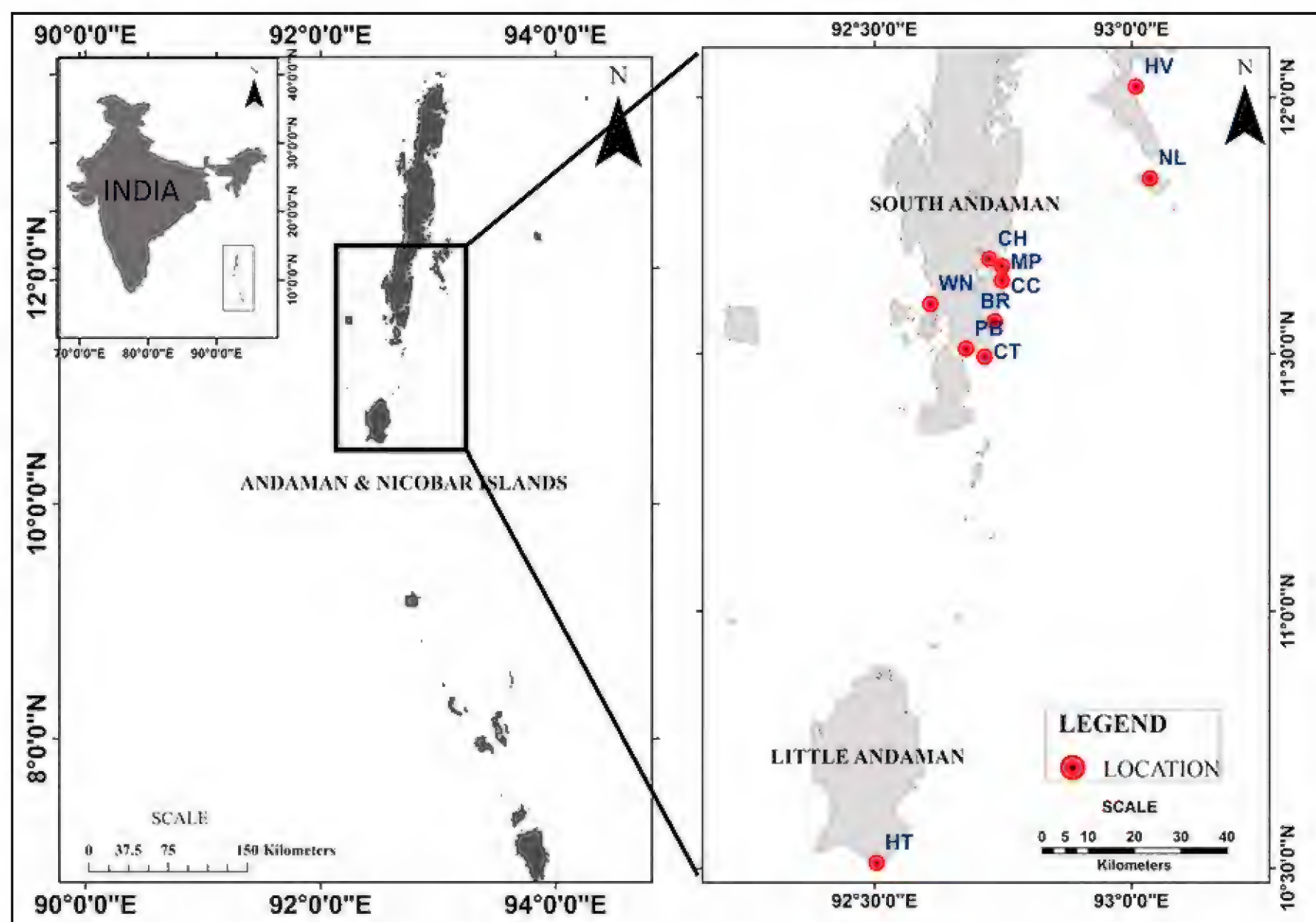


Figure 1. Study area of South Andaman Island (CT- Chidiyatapu; BN- Burmanallah; CC- Carbyn's Cove; MP- Marina Park; CH- Chatham; WN- Wandoor; PB- Pongibalu; HV- Havelock Island; NL- Neil Island; HT- Hut bay).

0.97) followed by Carbyn's Cove and Chidiyatapu whereas Marina Park showed the lowest value of evenness. The highest Margalef's diversity (d : 2.45) is observed at Chidiyatapu and Pongibalu and lowest being at Marina Park. In the present study, 24 species of amphipod were collected and studied viz. *Hyale honoluluensis*, *Parallorchestes* sp. (Hyalidae), *Corophium triaenonyx* (Corophidae), *Grandidierella gravipes*, *Grandidierella* sp.1 (Aoridae), *Ampelisca scabripes*, *Ampelisca* sp.1, *Ampelisca* sp.2 (Ampeliscidae), *Urothoe* sp.1, *Urothoe* sp.2 (Urothidae), *Atylus tridens*, *Polycheria osborni*, *Pardexamine* sp. (Dexaminidae), *Tiron* sp. (Synopidae), *Grandifoxux* sp., *Pontogeneia* sp. (Phoxocephalidae), *Paracalliopiella* sp., *Paramoera* sp., *Calliopius* sp. (Eusiroidea), *Melita* sp.1, *Melita* sp.2 (Hadzoidea), Caprellidae, Lysianassoidea, Haustoriidea. The Ampelescids *Ampelisca scabripes*, *Ampelisca* sp.1, *Ampelisca* sp.2 are abundant only

in Marina Park location where the substratum is fine sand. Whereas, Aoridae, Ampelescidae and Eusiroidea are found at all study locations. The most abundant families are the Ampeliscidae and the Eusiroidea. *Ampelisca scabripes* is by far the most abundant and widespread amphipod in the intertidal region of Andaman Islands. *Paracalliopiella* sp., *Paramoera* sp., and *Urothoe* sp. 1 were also found abundant during the period of study. *Grandidierella gravipes*, *Ampelisca scabripes*, *Paracalliopiella* sp., *Paramoera* sp. were observed in all the locations. Species diversity is highest in the Ampelescidae, Dexaminidae and Eusiroidea, (each 3 genera). Correlation analysis showed no strong relationship between benthic diversity indices and abiotic variables. However, there was a significant positive correlation between dissolved oxygen and Shannon-Weiner diversity ($r = 0.370$; $p < 0.05$).

A first approach on the molecular phylogeny of Cyamidae (Amphipoda Senticaudata Caprelloidea)

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ABSTRACT

A phylogenetic analysis of the family Cyamidae based on mitochondrial DNA sequences is proposed in the present study. Sequences of 16S and COI gene fragments were used to infer phylogenetic trees of the family, which showed partial congruence.

KEY WORDS

Whale lice; phylogenetic analysis; COI; 16S.

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INTRODUCTION

The family Cyamidae includes 27 species distributed in seven genera (Iwasa-Arai, 2017). All species are obligatory ectoparasites of marine cetaceans, and, for that reason, they are known as whale louse. Cyamidae belongs to the superfamily Caprelloidea, as previous works detected the common ancestry of cyamids and caprellid-like amphipods (Laubitz, 1993). However, there are only a few molecular analyses of the group until now (Kaliszewska et al., 2005; Callahan, 2008), especially due to the age of material, since most of the whale lice were collected during the whaling period (Margolis et al., 2000). Taking this into account, the present study is one of the first attempts to propose phylogenetic analyses of the family Cyamidae based on mitochondrial DNA sequences.

MATERIAL AND METHODS

Specimens of whale lice collected from several

cetacean species were mostly fixed and preserved in ethanol 70%, with some specimens from collections being fixed in formalin. *Caprella penantis* was used as outgroup. Total genomic DNA was obtained using a modified CTAB extraction (Gusmão & Solé-Cava, 2002). A fragment of the 16S ribosomal gene was amplified using the universal primers AMPH1F (GAC GAT AAG ACC CTAAAA GC – modified to Cyamidae) and AMPH2 (CGC TGT TAT CCC TAA AGT A) (France & Kocher, 1996), and a fragment of the COI gene was amplified using the primers COH1b (TGT ATA RGC RTC TGG RTA RTC) (Schubart et al. 2009) and the specific primer Cya1F (GCN GTY TTY GGD ATT TTY GC), designed herein. The sequences obtained were edited using SEQMAN II 4.0 (DNASTAR Inc.), aligned by the Clustal W algorithm implemented in MEGA6 (Tamura et al., 2011) and checked manually for misalignments. Cytochrome c oxidase sequences of *Cyamus kessleri* were obtained from GenBank. Phylogenetic trees for each gene were inferred by Neighbor-Joining, using the distance model K2P in the program MEGA.

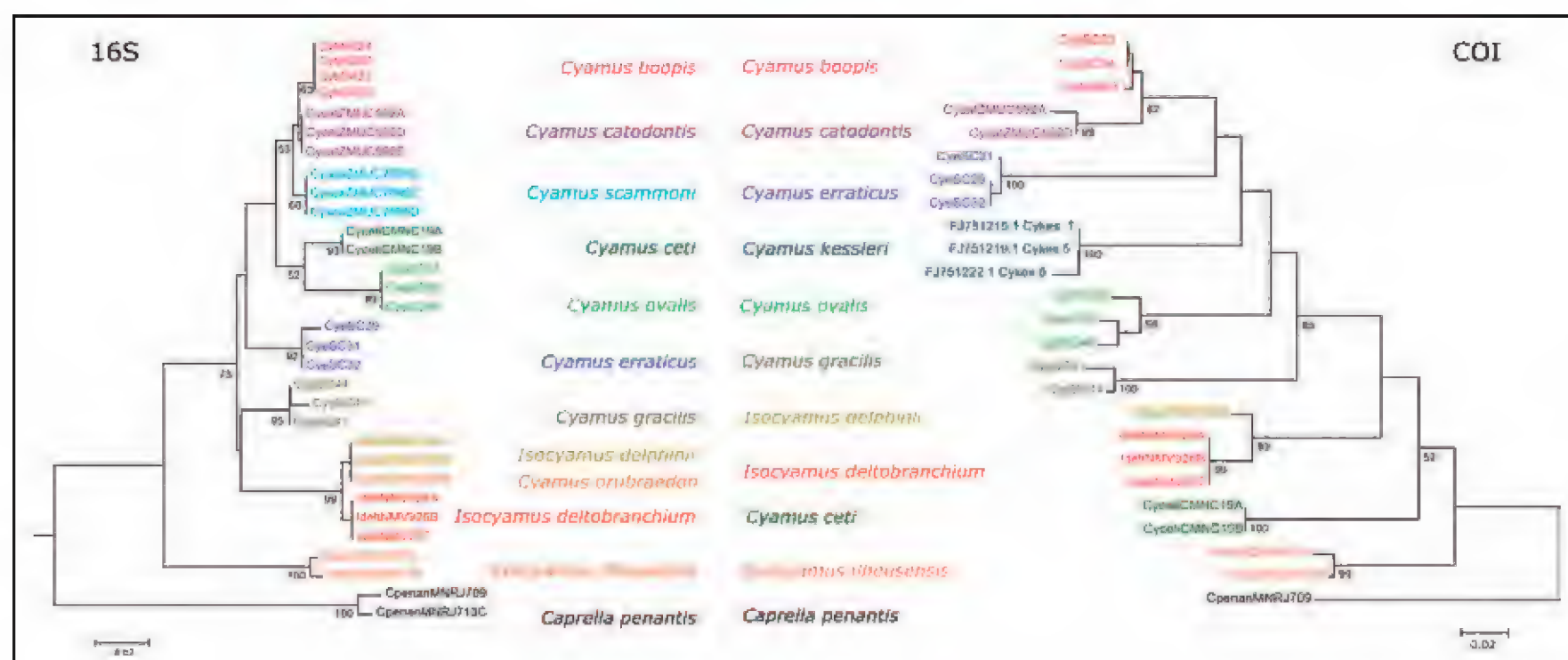


Figure 1. Neighbor-joining tree of Cyamidae, with K2P distance, of the 171bp fragment of 16S gene and the 191bp fragment of COI gene. Values below branches correspond to bootstrap.

RESULTS AND DISCUSSION

The results of the 16S and COI trees showed the family Cyamidae as a monophyletic clade, while the genus *Cyamus* appears to be paraphyletic and includes the genus *Isocyamus* (Fig. 1).

Cyamus boopis is the sister clade of *C. catodontis* in both gene trees, corroborating the previous morphological analysis (Haney, 1999). On the other hand, the clade *C. erraticus* (*C. boopis* + *C. catodontis*) observed by Haney (1999) only was corroborated by the COI tree. Other relationships between *Cyamus* were not recovered for both genes, and the COI tree topology did not agree with a previous COI tree inferred by Callahan (2008). Thus, the inclusion of genetic data from other Cyamidae species, together with the morphological set of characters, might elucidate the relationships within the group.

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Diversity and systematics of amphipods in Swiss rivers: River network structure shapes community structure

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ABSTRACT

We show that both native and non-native amphipod community assembly in river networks are influenced by the connectivity of habitats.

KEY WORDS

Amphipoda; Biodiversity; Community Assembly; Habitat Connectivity; Invasion Biology.

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Amphipods represent keystone species in many aquatic ecosystems and they contribute substantially to the biodiversity and functioning of macroinvertebrate communities in these habitats (Holsinger, 1976). Hence understanding their ecology and systematics is crucial for conservation measures. Especially in Europe there is a relatively high proportion of non-native amphipod species reaching new habitats (Bij de Vaate et al., 2002), and new conservation challenges arise. Furthermore, for the European Alps in general and Switzerland in particular, only limited information on amphipods has been available until recently (Altermatt et al., 2014).

Biological communities and hence patterns of biodiversity are shaped by habitat connectivity and dispersal of organisms. River networks offer unique possibilities to study these processes in a spatially explicit manner. Predictions from theoretical work imply that network structure has a major influence on measures of diversity (Carrara et al., 2012). Furthermore, experiments and data from fish or bacterial communities support these expectations (Muneepeerakul et al., 2008; Besemer et al., 2013). On a

global scale, rivers not only show these characteristic diversity patterns, but are also strongly affected by non-native species. They represent a major challenge for conservation biology and can have detrimental effects on native communities (Chandra & Gerhardt, 2008). Nevertheless it was rarely studied how the river network structure shapes the occurrence of non-native species. Here we present results on the spatial distribution of amphipod communities throughout Switzerland. We specifically studied the spatial imprint of the river network structure on communities of native and non-native amphipod species in Switzerland.

We collated an extensive database on amphipod occurrences in Switzerland, covering over 1,700 sites and 41 species and its underlying river network. Data origin from published literature, the biodiversity monitoring program of Switzerland (BDM coordination office, 2009), grey literature such as reports from environmental agencies and our own extensive sampling. Community data were analyzed as presence-absence matrix and summarized in 1000 km² subcatchments. Our analysis in the river Rhine drainage revealed distinct

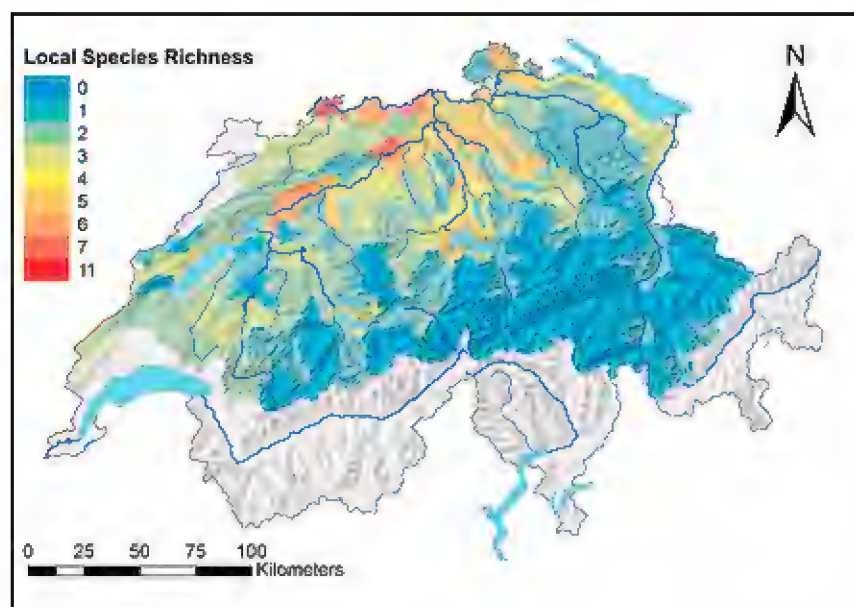


Figure 1. Amphipod richness in the river Rhine drainage in Switzerland. Data source: swisstopo (Art. 30 GeoIV): 5704 000 000 / swissTLM3D 2014, (reproduced with permission of swisstopo / JA100119)

patterns of local species richness along the river network and different distributions of native and non-native amphipod species. Species richness increases along the network from headwaters to the outlet site (Fig. 1).

This implies sufficient levels of dispersal between amphipod communities along the network. But non-native species are still mostly restricted to larger and better connected streams, whereas small and more isolated headwaters represent refugium habitats for native species. Species turnover (β -diversity) of native species shows the classical distance decay between sites in the network. Their communities are less similar with increasing distance. This suggests that native species might be dispersal limited. On the other hand, similarity of non-native communities between sites in the network is not decreasing by distance. This implies a potential influence of habitat filtering or minor dispersal limitation on non-native amphipod diversity. The modularity of the network has a significant imprint on amphipod communities. Overall, the expected diversity patterns are only observed when taking into account both native and non-native spe-

cies. Furthermore, our results improve the knowledge of Swiss amphipods and our understanding of the influence of spatial connectivity on biodiversity and invasion processes in natural systems.

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Amphipods of Switzerland

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ABSTRACT

We present the first conclusive overview on amphipods of Switzerland, with distribution maps of all 43 species recorded so far.

KEY WORDS

Amphipoda; Switzerland; Diversity; Alps; Distribution maps.

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Switzerland represents a biogeographically interesting region. The Swiss Alps are the origin of four major drainage areas in Central Europe. Hence knowledge of the flora and fauna of this region are of high interest. Amphipods as keystone species are no exception in this. But although the ecological importance and widespread distribution of amphipods is recognized, the actual diversity and distribution is still poorly studied in many areas, particularly in Switzerland. For example, up to now there exists no detailed overview of all amphipod species from Switzerland, no identification key covering all present drainage areas and especially very little information on amphipods in alpine and higher elevation habitats.

Over the last few years we collated an extensive database on amphipods of Switzerland. It covers more than 1,800 sites and more than 175,000 identified amphipod specimens. The data originate from various sources, such as the governmental biodiversity monitoring program, official reports, published literature and our own extensive sam-

pling during the years 2012 until now. All available data from collections in Switzerland were included. We present the results as distribution maps of all present species of Switzerland. Additionally we updated the checklist of Swiss amphipods from Altermatt et al. (2014). Over the last 150 years, 43 species were recorded in Switzerland. 21 of these were subterranean species (*Niphargus* sp. and *Crangonyx subterraneus* Spence Bate, 1859), and another 13 species should be considered non-native to Switzerland. A monograph on Swiss amphipods is in preparation and will be published soon (Altermatt et al., in prep.). The data will help to understand the ecology of Swiss amphipods, the influence of connectivity on amphipod distribution and diversity and the early detection of non-native species.

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Phenology of the locomotor rhythm in adults, juveniles, males and females of *Orchestia gammarellus* (Pallas, 1776) (Crustacea Amphipoda)

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ABSTRACT

Freshly collected individuals of *Orchestia gammarellus* (Pallas, 1766) (Talitridae) from the supralittoral zone of Ghar El Melh lagoon (N Tunisia) were housed in spring in a controlled environment cabinet. Locomotor activity rhythm of this species was recorded in spring, at a constant temperature. In the first experiment, juveniles and adults specimens were kept under light-dark cycle in phase with the natural diel cycle. In the second experiment males and females were maintained under constant darkness. According to double-plotted actograms, waveforms and periodogram analysis, results revealed different locomotor patterns. Locomotor rhythm of juvenile individuals was more stable than that of adults. Furthermore, results showed no significant differences between locomotor rhythm pattern of males and females.

KEY WORDS

Lagoon; locomotor rhythm; males; females; adults; juveniles.

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INTRODUCTION

The littoral environment is inhabited by many forms of arthropods, each characterized by particular ecophysiological requirements. It has been known that supralittoral amphipods are mainly nocturnal animals, whereas *Orchestia montagui* Audouin, 1826 (Talitridae) collected in the Northern of Tunisia exhibited a diurnal locomotor behavior (Jelassi et al. 2013; Ayari et al. 2015, 2016).

Locomotor activity may differs between sexes, and studies have found that males of *Drosophila melanogaster* show a steady and stereotyped walking pace, while females modulate their activity patterns (Martin et al., 1999). Bahndorff et al. (2012) demonstrated that males of *Musca domestica* were more active than females. Ayari et al.

(2016) showed that locomotor rhythm of males of *O. montagui* was more stable than that of females of the same species. Moreover, the study of locomotor activity of *Orchestoidea tuberculata* Nicolet, 1849 showed a difference in behavior between adults and juveniles. These behavioral differences would be a means of juveniles' protection against cannibalism (Kennedy et al., 2000).

This study aimed to characterize the effect of age and sex on the locomotor rhythm parameters of *O. gammarellus* (Pallas, 1766).

MATERIAL AND METHODS

Specimens of *O. gammarellus* were collected during spring, from the supralittoral zone of Ghar El

Melh lagoon (37°10'N, 10°11'E). Collected animals were quickly transported to the laboratory in Perspex boxes. Two experiments were performed. Firstly, 15 adult males (body length= 13.4 ± 0.97 mm) and 15 juvenile males (body length= 8.3 ± 1.25 mm) were used to test whether the locomotor activity rhythm of juveniles was different from those of adults. Secondly, adult males (n= 15) and adult females (n= 15) were run simultaneously to test the effect of sex on locomotor rhythm. Males were easily distinguishable from females by their highly developed gnathopod I and pereopod VII. For each of these experiments, animals were housed to the actographs containing humid substratum and a small amount of carrot as food. Then, specimens were kept in a controlled environment chamber maintained at a constant temperature of 18 ± 0.5 °C. Locomotor activity in each actograph was provided with infrared beam focused across the platform. Interruptions due to animal activity were recorded by a data-logger using the Sand 16 software program and downloaded on a computer every 20 min. The recording apparatus and controlled environment chamber were designed and constructed in the School of Biosciences University of Birmingham, Birmingham, UK.

RESULTS AND CONCLUSIONS

Under constant darkness, our results revealed the presence of a circadian and ultradian endoge-

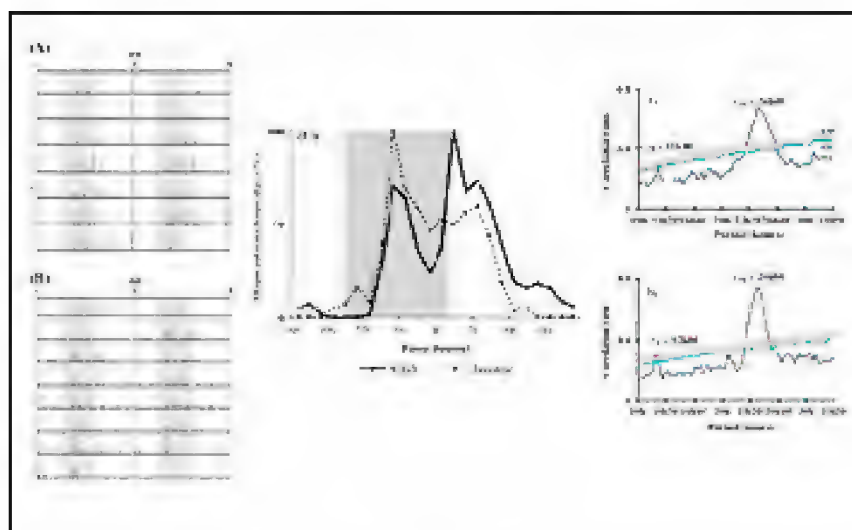


Figure 1. Double-plotted actograms selected to illustrate the activity rhythm pattern in adult (A) and juvenile (B) under natural light-dark cycle. Each actogram is associated with a superposed mean hourly activity curve (C) and periodogram (a1) and (b1), respectively, 0.05, 0.01 and 0.001% represent the three levels of significance. Gray area correspond to the experimental night (LD 14h34:9h26).

nous component for this species, with periods close to 24 and 12 h, respectively. Generally, a sensitivity to the subjective sunrise or/and sunset was observed (Fig. 1).

Both adults and juveniles exhibited circadian locomotor rhythmicity under nLD cycle; however juvenile individuals were more active than adults and had a better defined locomotor rhythm. On the other hand, the most important adult's activity peak occurred around the experimental sunrise, whereas for the juveniles, this peak occurred during the night. In fact, juveniles, which were more vulnerable than adults, required a higher humidity to avoid desiccation.

Despite the absence of the significant difference between locomotor behavior of males and females, circadian period and locomotor rhythm stability were, respectively, longer and better defined for males than for females. This sexual variation of activity pattern was demonstrated in *M. domestica* (Bahndorff et al., 2012) and in *O. montagui* (Ayari et al., 2015).

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Unexpected diversity of the deep sea wood-associated amphipod *Bathyceradocus* Pirlot, 1934 (Amphipoda Maeridae)

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ABSTRACT

Sunken woods, organic falls or plant remains are in the Deep-Sea an original source of nutrition. More than large organic inputs to the seafloor, these unique ecosystems are considered as ephemeral but inhabited by specialized fauna also represented by amphipods. There are only 3 species of *Bathyceradocus* Pirlot, 1934 (Maeridae) known and described as wood-associated species. A total of 141 specimens have been recently collected from several cruises of the Tropical Deep Sea Benthos programme and during the KuramBio I expedition. Morphological and molecular investigations (COI and 18S) have provided amazing results suggesting an unexpected diversity for this group of rare and atypical amphipods. Molecular species delineation results indicated more than 15 species suggesting a considerable number of new ones. Morphological characters have to be redefined according to this new collection in order to revise the genus *Bathyceradocus*.

KEY WORDS

Sunken woods; Deep-Sea; *Bathyceradocus*.

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INTRODUCTION

The presence of animals on plant remains at the deep-sea floor was first documented by the Challenger expedition (1872-1876). The Galathea expedition (1950-1952) also recorded the abundant presence of plant remains on the deep-sea floor (Bruun, 1959). The importance of plant material in the deep sea was first emphasized by Wolff (1979). The fauna associated with these remains is unique. Apart from sporadic taxonomic considerations, plant associated organisms were seen as zoological and/or ecological curiosities and thus only anecdotally studied. But this habitat was recently emphasized as deep-sea environment of major evolutionary importance. The zoological affinities

of organisms associated with sunken woods and whale falls with those from hydrothermal vent or cold seep ecosystems suggest that these ecosystems, developing on organic substrata in the deep-sea, could be an evolutionary step toward the colonization of hydrothermal vents (the so-called “wooden steps” hypothesis of Distel et al. (2000)). However, still not much data on wood-associated organisms are available. Amphipods are also inhabitant of sunken wood ecosystems. Several species, belonging to the genera *Onesimoides* Stebbing, 1888, *Paronesimoides* Pirlot, 1933 and *Exitomelita* Tandberg, Rapp, Schander, Vader, Sweetman et Berge, 2011, had already been mentioned in their taxonomic descriptions as found/living on sunken woods. Furthermore, the genus *Bathyceradocus* Pirlot, 1934

(Maeridae) is composed of 3 known species, all collected from wood or plant remains, namely *B. stephensi* Pirlot, 1934 described from three specimens collected during the cruises of the SI-BOGA in the Celebes Sea (Pirlot, 1934), *B. iberiensis* Andres, 1977 collected from Atlantic (Andres, 1977), and *B. wuzzae* Larsen et Krapp-Schickel, 2007 recently described by Larsen & Krapp-Schickel (2007) from the vicinity of Pacific hydrothermal vents (Juan de Fuca).

STUDIED MATERIAL

In 2004, the Tropical Deep Sea programme (ex MUSORSTOM, MNHN-IRD, <https://expeditions.mnhn.fr/program/tropicaldeep-seabenthos>) started a series of cruises devoted to the exploration of the sunken wood environments. Since the first MUSORSTOM cruises in the Philippines (Forest, 1981), it became clear that the slopes of oceanic islands within these regions accumulate large amounts of decomposing vegetation. Subsequent cruises in the Solomon Islands, Vanuatu Archipelago and Papua New Guinea specifically targeted sunken vegetation to further characterize its fauna. Several deep sea cruises provided 118 *Bathyceradocus* specimens in the depth range between 400–3500 m from different realms (mainly Central Indo-Pacific but also Western Indo-Pacific and Tropical Atlantic) corresponding to 15 deep-sea cruises and more than 51 sampling stations. Additionally, during the KuramBio I expedition, which was devoted to study the abyssal of Kuril-Kamchatka area, a few pieces of sunken wood were found. One of them collected at the depth of 5200 m harbored 23 amphipods of the genus *Bathyceradocus*.

RESULTS AND CONCLUSIONS

Taxonomic and phylogenetic studies have been conducted on this collection. The first morphologi-

cal hypothesis suggested that the specimens from Indo-Pacific and Atlantic waters show some similarities with *B. stephensi*. On the other hand the North-Pacific (Kuril-Kamchatka) specimens apparently belong to undescribed species. Molecular analyses (COI and 18S) showed that this group is much more diversified than expected: 15 species (based on molecular species delineation) have been evidenced in the studied samples revealing a cryptic diversity in this genus. Morphological examinations indicated that the diagnostic characters previously defined for the *Bathyceradocus* species need to be revised by integrating this new material, in order to describe new species. The taxonomical context has to be settled in order to propose hypotheses on biogeography and wood colonization patterns of the *Bathyceradocus* group.

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Preliminary results on Amphipoda diversity from the MADIBENTHOS expedition in Martinique (Lesser Antilles)

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ABSTRACT

MADIBENTHOS expedition was an extensive survey of the coastal marine biodiversity in Martinique. An important collection of amphipods has been sampled and preliminary results suggest an impressive diversity (for now 119 species) by comparison with the data on the Caribbean Sea.

KEY WORDS

Lesser Antilles; Caribbean sea; diversity; conservation.

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INTRODUCTION

The MADIBENTHOS expedition led by the Muséum National d'Histoire Naturelle (MNHN) was a 6 weeks coastal survey (September-October 2016) in Martinique (Lesser Antilles), with the main objective of collecting baseline data of marine benthic invertebrates in a purpose of conservation. The scientific objectives of the expedition were:

(1) Discovery and Exploration: documenting unknown species;

(2) Macroecology: understanding patterns of habitat and species richness and diversity in complex ecosystems;

(3) Data Enrichment: feeding new data into international databases. The purpose of this “in progress” study is to emphasize the large collection of amphipods collected during the MADIBENTHOS expedition, which would be one of the first large scale inventory of this group in the Caribbean Sea.

MATERIAL AND METHODS

The deployment on the field of 62 participants (experienced divers, taxonomists, naturalists and collection managers) from several french and international research institutes allowed a large scale prospection of the diverse marine habitats of Martinique. A total of 500 sampling stations were studied from intertidal to the maximum depth of 100 m depth with a panel of different sampling methods. Two main under-water devices operated by scuba-divers were the more efficient for amphipod sampling. The suction sampler (Fig. 1A) consists of a 2 m long tube connected to a source of compressed air that empties into a 1 mm mesh-size net. The suction sampler is operated on soft seafloor sediments, to collect the upper 1–2 cm of material and the thin layer of ooze and microalgae found in rock crevices. A suction sample typically covers 1 to 2 m² of seafloor and generates 2–15 l of bottom material. The brushing basket (Fig. 1B) consists

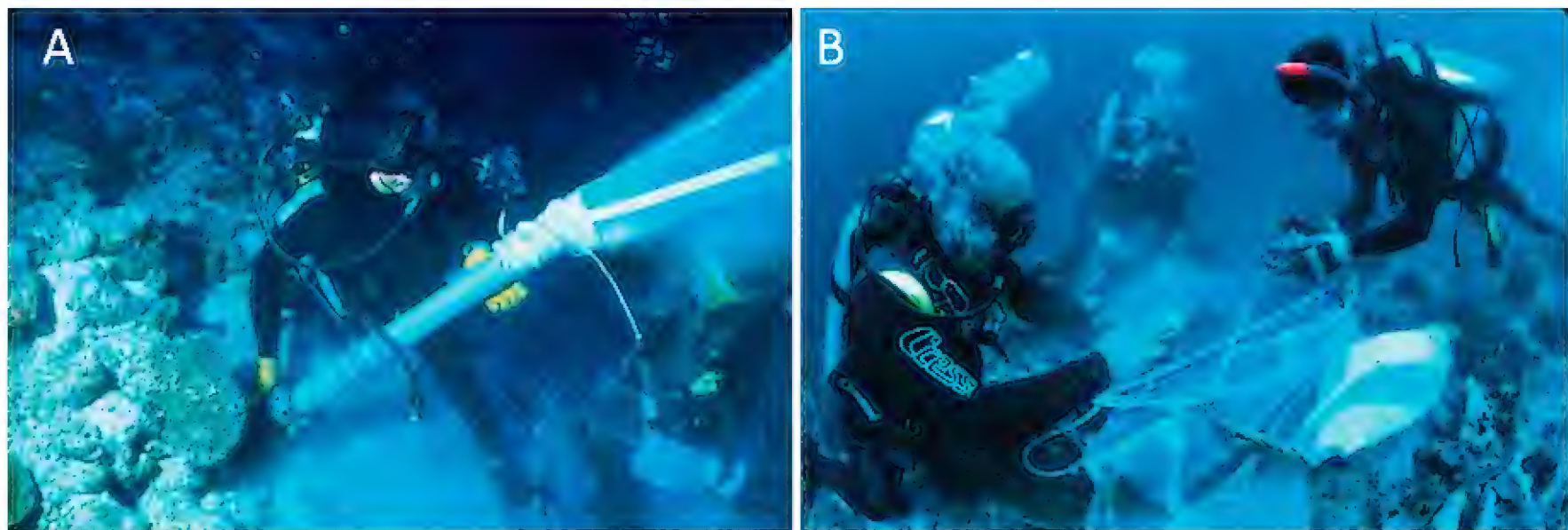


Figure 1. Scuba-diving devices. A Suction sampler. B Brushing baskets. Photos Madibenthos expedition. Copyright MNHN.

of a 1 mm mesh-size net protected inside and outside by sturdy laundry baskets. Loose rocks and coral rubble are placed in the basket and vigorously brushed by hand. The cleaned rock is returned to its place and the residue accumulates in the net. Alternatively, and especially in deeper water (30–60 m) with limited SCUBA autonomy, the basket can be filled with loose rocks and lifted to the surface where the rocks are then carefully brushed. A brushing sample typically covers 1 to 2 m² of seafloor and generates 1–5 l of bottom material. A light semi-circular dredge (opening width: 30 cm, height: 20 cm) was also deployed from a small boat on sediment patches in 2–100 m. Bulk samples and sediment residues were sieved in fresh seawater and fractioned through a set of sieves from 10 to 0.5 mm, and the light (containing i.e. polychaetes and peracarids) and heavy fractions (containing sediment and shells) were separated. The coarse fractions were sorted with the naked eye, the smaller ones (< 3 mm) under a dissecting microscope.

RESULTS AND CONCLUSIONS

Amphipods occurred at 168 sampling stations, ranging from intertidal to 100 m depth (Fig. 2), mainly at brushing stations (37 %), dredging stations (35 %) and suction stations (18 %). The study of this large amphipod collection has been initiated during a recent taxonomic workshop organized by MNHN on March 2017 in order to identify specimens up to the species level.

A total of 32 stations have been processed, representing a total of 1525 individuals identified to species level. Seven stations are in progress, separated at family level (814 individuals) and 129 stations remain to be checked. At this time, 119 species have been identified belonging to Gammaridea (54 sp.) and Senticaudata (65 sp.). The most speciose families are Leucothoidae (17 species) and Maeridae (15 species), followed by Aoridae (9 species), Ampeliscidae (8 species), Photidae (8 species) and Caprellidae (7 species). For now, the most speciose and abundant station is the brushing station AB358 (Southern Martinique: Grande Anse du Diamant; 17 m depth) with 16 families, 27 species and 206 individuals, mainly belonging to Chevaliidae (30.1 % of total abundance, with one dominant species *Chevalia* sp. nov.), Leucothoidae (21.4 %), Photidae (12.1 %) and Ampeliscidae (11.7 %). Comparisons will be performed between Caribbean and Atlantic stations.

These preliminary results suggest a rather large percentage of new species (about 20 %) and the expected species richness will be probably greater than the data reported in the literature for known Caribbean areas. For example, Martin et al. (2013) reported a total of 73 amphipod species for Lesser Antilles. However, when considering exclusively benthic and shallow water (≤ 100 m) data of their work, only 44 species are present in that area. The preliminary results of the MADIBENTHOS expedition suggest that this new inventory will spread out our knowledge of amphipod diversity in Lesser Antilles.

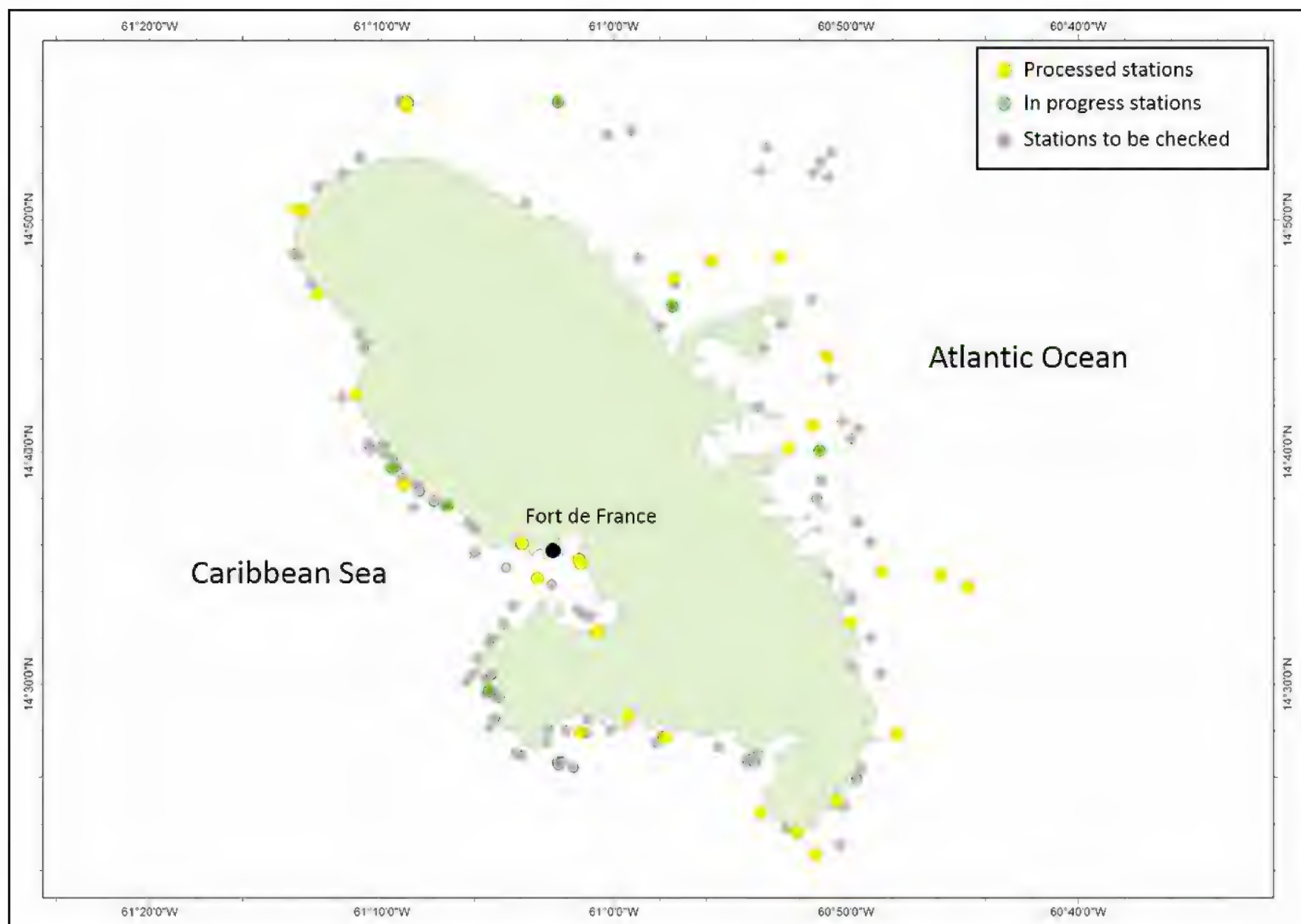


Figure 2. Geographical location of benthic sampling stations all around Martinique Island (Lesser Antilles) during the MADIBENTHOS expedition.

ACKNOWLEDGEMENTS

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Philippe Bouchet), with funding from the European Regional Development Fund (ERDF), the Territorial Collectivity of Martinique (CTM).

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New species and new records of *Quadrimaera* Krapp-Schickel et Ruffo, 2000 (Amphipoda Maeridae) to Brazil

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ABSTRACT

Quadrimaera Krapp-Schickel et Ruffo, 2000 (Amphipoda Maeridae) is a cosmopolitan genus typical from tropical shallow waters. The genus is composed by 34 species, five of them reported from Brazil. During samples conducted along the northeastern Brazilian coast, between 2010 and 2014 in intertidal and subtidal zones, two new species of *Quadrimaera* specimens were described. We also report the distribution extension range of *Q. rocasensis* Senna et Serejo, 2007 and *Q. pieteri* Krapp-Schickel et Ruffo, 2000 to Brazilian waters and describe for the first time the female specimen of *Q. rocasensis*.

KEY WORDS

South Atlantic Ocean; *Quadrimaera pieteri*; *Q. rocasensis*; taxonomy.

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INTRODUCTION

The genus *Quadrimaera* Krapp-Schickel et Ruffo, 2000 is a cosmopolitan group including 34 species, most of them typical from tropical shallow waters (Krapp-Schickel, 2000). The type species of the genus was originally described as *Gammarus quadrimanus* Dana, 1852. One century later, with the establishment of the Family Melitidae, the species was renamed *Maera quadrimana*. Rising from the heterogeneity of the *Maera* Leach, 1814 genus, the *Quadrimaera* genus was established. This genus is unique by presenting: (1) mandibular palp article 3 subequal in length or longer than article 2; (2) gnathopod 2 palmar corner about 90%; (3) pereopods with bifid dactyl.

In the present work, we describe, preliminarily, two new species of *Quadrimaera* from the Brazilian

coast. Beside this, we report distributional extensions and discuss the taxonomy of the species *Q. rocasensis* and *Q. pieteri*.

MATERIAL AND METHODS

Amphipods were collected along the northeastern Brazilian coast between 2010 and 2014 in intertidal and subtidal zones. The specimens were fixed in 70% ethanol. For the new species, the male holotypes were dissected under stereoscope microscope. Mouthparts and appendages were mounted on permanent slides with glycerol gel. For the new records, the habitus was drawn under stereoscope microscope with a camera lucida, while the diagnostic dissected appendages were drawn under compound microscope with a camera lucida.

RESULTS

Quadrimaera n. sp. 1

TYPE LOCALITY. Pirangi Beach, Rio Grande do Norte State, Brazil.

DIAGNOSIS. Antenna 1 and 2 flagellum setose. Accessory flagellum 6-articulated. Coxa 1 posteroventral corner rounded. Article 2 of mandibular palp longer than others. Palm gnathopod 2 transverse with 7 concavities, three most proximal are U-shaped with the last one deeper than the others; the others four distal are elliptical, being the anterior most and the distalmost deeper than the two intermediary; dactyl inner margin regular with sparse setae. Pereopods 5–7 basis slender with pronounced posterodistal lobe. Telson cleft about 75% of its length, lobes with two notches and 5 robust setae each one.

Quadrimaera n. sp. 2

TYPE LOCALITY. Dois Coqueiros Beach, Ceará State, Brazil.

DIAGNOSIS. Antenna 1 accessory flagellum with 7 articles. Antenna 2 cone gland not reaching the end of article 3. Eyes rounded. Coxa 1 posteroventral corner rounded produced. Gnathopod 1 carpus longer than propodus, posterior margin beset with plumose setae. Gnathopod 2 propodus palm with a medial excavation and a molar shaped hump, dactyl inner margin acutely produced. Telson deeply cleft (about 70%), telsonic lobes truncate with five stout setae longer than telson.

Quadrimaera pieteri Krapp-Schickel et Ruffo, 2000

EXAMINED MATERIAL. 1 adult male, Parque Nacional Pedra da Risca do Meio, Ceará State, Brazil. 1 adult male, Boipeba Beach, Bahia State, Brazil.

DESCRIPTION. Material from Northeastern Brazil: Coxa 1 posteroventral corner acute and lightly produced. Gnathopod 2, carpus reduced, subtriangular; propodus enlarged, propodus palm ornamented with a clear central U-shaped excavation, preceded by a proximal subtriangular process and proceeded by a distal subrectangular process, palm defined by an acute process, preceded by a U to V-shaped excavation; dactyl inner margin weakly to strong

sinuosity. P7 basis posterior margin strongly globose; propodus posterior margin with a tuft of long setae. U2 peduncle slightly longer than rami; outer and inner ramus similar in length. U3 peduncle shorter than rami, outer ramus longer than inner, with two sets of setae.

DISTRIBUTION. Lesser Antilles, Caribbean Sea (type locality). Parque Nacional Pedra da Risca do Meio, Ceará State, Brazil. Pirangi Beach, Rio Grande do Norte State, Brazil. Boipeba beach, Bahia State, Brazil (analyzed material). Prainha, Arraial do Cabo, Rio de Janeiro State, Brazil.

REMARKS. The specimens of *Quadrimaera pieteri* here analyzed differ from material from the type locality by having: (1) the P7 posterior margin that is lightly more globose; (2) U2 peduncle slightly longer than rami (while similar in length in specimens from the type locality). These specimens are also very similar to *Quadrimaera pieteri* Krapp-Schickel & Ruffo, 2000 from Rio de Janeiro, Brazil especially by the gnathopod 2 propodus palm variations and the U3 outer ramus sets of setae. Considering that the variations found in Brazilian specimens in comparison to Caribbean specimens are not sufficient to separate both species, it is more prudent to consider all of these Brazilian specimens as belonging to the same species.

Quadrimaera rocasensis Senna et Serejo, 2007

EXAMINED MATERIAL. One adult female, Pirangi Beach, Rio Grande do Norte State, Brazil. 1 adult female, Boipeba Beach, Bahia State, Brazil.

DESCRIPTION. Female (sexual dimorphic characters): coxa 1 posteroventral corner rounded, anterior margin lightly projected. Gnathopod 1 basis longer than ischium and merus together, posterior margin with 7 slender setae; carpus facially setose, more than males; propodus ovate, facially setose, propodus palm with minute setae; dactyl inner margin naked. Gnathopod 2 basis anterior margin with stout setae, anteriorventral corner rounded and produced, posterior margin with two slender setae; carpus reduced, subtriangular; propodus robust, subrectangular, with setae on both margins, propodus palm setose with one U-shaped concavity preceded by a broad and truncate process and proceeded by an acute and pronounced process that define the palmar corner; dactyl inner margin with a delicate

projection. Telson deeply cleft, 0.5 times longer than wide, lobes apically notched, with four slender and bifid setae.

REMARKS. *Quadrimaera rocasensis* is originally described from Atol das Rocas Island, Brazil. The present study reports the species from the mainland, being found in Pirangi Beach, Rio Grande do Norte State and Boipeba Beach, Bahia State. The female specimen hitherto unknown to science is firstly described.

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New species of *Stenothoe* Dana, 1852 (Amphipoda Gammaridae) for the Northeastern Brazil

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ABSTRACT

A new species of *Stenothoe* Dana, 1852 (Gammaridae) is described to Northeastern Brazil, where the diversity of this genus is poorly known. The new species is unique by having: (1) the male Gnathopod 2 propodus palm with two acute and subtriangular processes, the posterior one smaller than other one; (2) pereopod 5 basis subrounded; (3) Uropod 3 with serrate tips. This is the second species of the genus described to Brazilian Coast.

KEY WORDS

Gammaridea; South Atlantic Ocean; Amphipoda; taxonomy.

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INTRODUCTION

Stenothoidae is a monophyletic family defined by having antenna 1 with nasiform process, coxae 3 and 4 expanded covering the others and urossome partially fused (Krapp-Schickel & Koeneman, 2006). This family includes almost 200 species in about 40 genera (Krapp-Schickel & Koeneman, 2006; Krapp-Schickel, 2015).

Stenothoe Dana, 1852 is the most diverse genus of the family Stenothoidae, presenting a worldwide distribution (Krapp-Schickel, 2015). Nevertheless, only *Stenothoe valida* Dana, 1853 was described to Brazil until now (Wakabara & Serejo, 1998). *Stenothoe valida* is considered a cosmopolitan species, being recorded to Atlantic, Mediterranean, Indic and Pacific Ocean and to South Africa and Australia (Krapp-Schickel, 2015). Many morphological differences have been noted between the *S. valida* original description and the others records

throughout the world, suggesting that *S. valida* could be a complex (Krapp-Schickel, 2015).

In the present work, we describe preliminarily a new species of *Stenothoe* to Brazil, being the second species of the genus to this country and also the first record to genus to Northeastern Brazil.

MATERIAL AND METHODS

Amphipods were collected on subtidal zone, in Pecém Harbor, Ceará state, Brazil in April 2010. The specimens were fixed in 70% ethanol. For the new species, the male holotypes were dissected under stereoscope microscope. Mouthparts and appendages were mounted in permanent slides with glycerol gel. For the new records, the habitus was drawn under stereoscope microscope with camera lucida, while the diagnostic dissected appendages were drawn under optic microscopic with camera lucida.

RESULTS

Stenothoe n. sp.

TYPE LOCALITY. Adult Male, Pecém Harbor, São Gonçalo do Amarante municipality, Ceará state, Brazil

DIAGNOSIS. Rounded and big eyes. Maxilliped inner plate large and produced. Gnathopod 2 isquium subtriangular, propodus palm with a rounded process near hinge of dactyl, proceeded by a pronounced and acute one, dactyl robust, narrowing abruptly. Coxae 3 subrectangular with subparallel margins. Pereopod 6 and 7 with a regular merus. Telson with 5 lateral setae.

DESCRIPTION. Ocular lobe triangular. Antenna 1 longer than Antenna 2; flagellum with 14 articles. Antenna 2. Maxiliped outer plate rounded, short, about 0.3 times longer than inner plate; inner plate subrectangular, large, distal margin produced in an acute point, facial margin with a rounded process; palp articles 1 and 2 subquadrate, article 3 subrectangular, dactyl falcate beset with setae. Gnathopod 2, coxae 2 subrounded, ventral margin beset with 6 small setae, basis strong and scarcely setose, isquium and merus similiar in lenght, merus subquadrate, isquium subtriangular, carpus falcate and poorly developed, propodus long, large, palm with small undulations and 2 pronounced processes near hinge of dactylus, the posteriormost rounded and not produced, the anterior one produced and acute. Gnathopod 1 and 2 dissimilar in size and shape. Gnathopod 1 subchelate, poorly developed. Coxa 2 trapezoid, anterior margin straight, posterior margin rounded, ventral margin beset with small setae. Gnathopod 2 merus subquadrate, similar in length to ischium, carpus falcate, propodus palm straight, pointed with little undulated processes and two developed ones near hinge of dactyl, the distal one truncated and the anterior most acute, palmar margin beset with a dense fringe of long setae. Coxa 3 subrectangular, anterior and posterior margins subparallel. Pereopod 3 isquium not larger than others segments, propodus 0.1 times longer than carpus. Coxae 4 subtriangular, well developed. Pereopod 4 carpus 0.3 times the length of propodus. Pereopods

5–7 ischium well developed and large. Uropod 1 peduncle 0.27 times longer than rami, inner ramus slightly shorter than outer one. Uropod 2 rami 2-articulated, 0.3 times longer than peduncle.

REMARKS. The new species is included in *Stenothoe valida* complex by presenting: (1) Male gnathopod 2 propodus palm almost straight with two process near hinge of dactyl; (2) P5-7 merus enlarged. But the new species differs from *S. valida* by: (1) the pronounced and developed maxiliped inner plate; (2) the gnathopod 2 propodus palm; (3) the sub parallel margins of coxa 3; (4) the pereopods 6 and 7 enlarged isquium and (5) the telson marginal setae. *Stenothoe* n. sp. is also closed related with the species *S. marina*, but differs from it by the shape of gnathopod 1. The new species appears being near to *Stenothoe* sp. from Rio de Janeiro, described by Serejo (1998), but it's different by the gnathopod 2 propodus palm processes and the shape of P6 and P7. The species described by Serejo (1998) is probably a new species from the *S. valida* complex.

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Amphipod assemblages from *Posidonia oceanica* vary seasonally in Giannutri Island (Central Tyrrhenian Sea, Italy)

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ABSTRACT

Data from a one-year survey of amphipod assemblages from a nearly undisturbed *Posidonia oceanica* meadow from Giannutri Island (Tyrrhenian Sea, Italy) are used to investigate the seasonal patterns in species abundance and composition.

KEY WORDS

Amphipoda; seasonality; Giannutri Island; Mediterranean Sea.

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INTRODUCTION

Seasonal fluctuations are thought to play a relevant role in both defining species abundance within communities and promoting species coexistence, with direct and indirect mechanisms involved in controlling diversity and composition of ecological communities through time (Shimadzu et al., 2013). Seasonal studies are especially needed in the marine environment, where the seasonal patterns may differ from those recorded on land (Shimadzu et al., 2013).

We carried out a yearly survey of amphipod assemblage structure from a nearly undisturbed *P. oceanica* meadow located in Giannutri Island (central Tyrrhenian Sea) to highlight possible seasonal changes.

MATERIAL AND METHODS

The study area was a *Posidonia oceanica* meadow from Giannutri Island (central Tyrrhenian Sea,

Italy), which is part of the National Park of Tuscan Archipelago and a Special Area of Conservation (SACIT51A0024). Samples were collected by SCUBA diving at a constant depth between 15-20 m, using an air-lift sampler in February, May, August and November 2014, for a total of six samples per season. A two-way PERMANOVA and nMDS were performed to compare assemblages structure between seasons and the species that mostly contributed to dissimilarity among temporal clusters were identified by SIMPER.

RESULTS AND DISCUSSION

A total of 1,144 specimens were identified at the species level, belonging to 62 species and 24 families. Our results showed the presence of a pool of species (*Apherusa chiereghinii*, *Apolochus neapolitanus*, *Liljeborgia dellavallei*, *Dexamine spinosa* and *Apolochus picadurus*) found throughout the whole year in the studied *P. oceanica* meadow, in agreement with other observations from the Medi-

terranean Sea (Zakhama-Sraieb et al., 2011; Scipione & Zupo, 2010; Bellisario et al., 2016).

No differences were found on amphipod assemblages between spring and summer (pairwise comparison, $F = 1.013$; $p = 0.104$), while winter and autumn assemblages differed among themselves ($p < 0.05$) and from the spring-summer season ($p < 0.05$ in all cases). Such pattern allowed identifying three main seasonal groups, represented by autumn, winter and the warm season (i.e., spring and summer). During the warm season, assemblages were characterized by a high number of individuals partitioned in a relatively high number of species, thus causing a significant contraction in terms of species dominance. During the autumn, the structure of amphipod assemblages became dominated by two species, *Socarnes filicornis* and *Gammarella fucicola*. Winter showed the lowest values in terms of species diversity and abundance, and two weakly associated species, *Leucothoe euryonyx* and *Deflexilodes subnudus*.

The observed seasonal changes are likely related to direct and indirect effects of the seasonal patterns of *P. oceanica* life cycle. The high number of species observed during the warm season may be due to a more efficient partitioning of the abundant resources available at that time, when seagrasses reach their highest productivity showing a peak in epiphyte assemblages, fostering a reduction in trophic niche overlap (Michel et al., 2015). During the autumn, species as *G. fucicola* can be advantaged, being able to accomplish the whole biological cycle within the seagrass litter which is abundant in this season (Lepoint et al., 2006). During the winter, the seagrass minimum productivity, the high hydrodynamic regime and the sparse canopy due to short leaves may strongly affect the removal of leaf litter and the settlement of individuals, likely explaining the lower diversity of the winter amphipod assemblages.

Our results reinforce the view that the relationships between *P. oceanica* and the associated amphipods may imply multiple and interactive factors which mould local community through the year (Sturaro et al., 2015).

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Patterns of amphipod distribution in *Posidonia oceanica* from the Mediterranean Sea

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ABSTRACT

In this note we report the results from a meta-analysis on the distribution of amphipods in *Posidonia oceanica* meadows, showing well-defined ecological mechanisms of geographic subdivision in the Mediterranean Sea.

KEY WORDS

Posidonia oceanica; Macroecology; Beta-diversity

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INTRODUCTION

Recent findings showed how communities are structured by a combination of processes acting at different spatial scales, thus driving attention on the geographic scale of variation (Moritz et al., 2013, Bellisario et al., 2016). Here we studied the distribution of amphipods on *Posidonia oceanica* meadows over the whole Mediterranean Sea to test if communities observed in various zones of the basin differ in composition, diversity and structuring mechanisms.

MATERIAL AND METHODS

An extensive review of current and past literature was conducted in order to extract all available information about the distribution of amphipods on *P. oceanica* across the Mediterranean basin (Fig. 1). Specific variables were downloaded for each locality: average Sea Surface Temperature and Salinity (<http://marine.copernicus.eu>), inorganic load, acidification, pollution and fishing pressure (<https://www.nceas.ucsb.edu/globalmarine>). Vari-

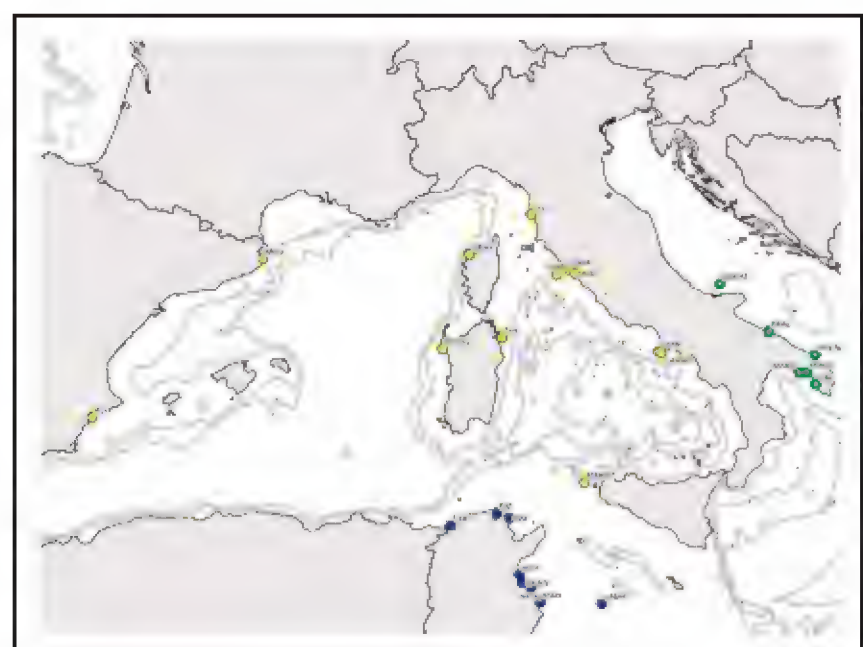


Figure 1. Sampling sites.

ables were combined with geographic data through a PCNM analysis and a dbRDA was carried out to highlight amphipod community structure. Also, beta-diversity partitioning was used to investigate for mechanisms of amphipod diversity, by measuring the Jaccard index (β_{jac}) partitioned into turnover (β_{tu}) and nestedness (β_{jne}) components (Baselga, 2010).

Crustaceans associated with the loggerhead sea turtles in the central Mediterranean Sea

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ABSTRACT

The biodiversity of *Caretta caretta* crustacean epibionts in the southern Tyrrhenian Sea (Mediterranean Sea) were analysed and one new record for the loggerhead turtles was reported.

KEY WORDS

Epibiont; crustaceans; *Caretta caretta*; central Mediterranean Sea.

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INTRODUCTION

The loggerhead turtle *Caretta caretta* (Linnaeus, 1758) is the most common sea turtle species in the Mediterranean Sea and plays an important role in the marine ecosystem (Foti et al., 2009). *Caretta caretta* is colonized by diversified communities of epibionts (Frick et al., 2000); they may benefit from increased survival, foraging, and dispersal, compared with those on inanimate flotsam (Karaa et al., 2012), developing a variable degree of association with living organisms (Frick & Pfaller, 2013).

In the Mediterranean Sea *C. caretta* carapace provides a microenvironment for several groups of animals, particularly crustaceans (Kitsos et al., 2005; Badillo, 2007; Sezgin et al., 2009; Zakhama-Sraieb et al., 2010; Casale et al., 2012; Blazewicz-Paszkowycz et al., 2012; Domenech et al., 2014). The present study examined the occurrence of crustacean assemblages on loggerhead turtles collected in the central Mediterranean Sea, in order to improve knowledge about the structure and composition of epibiont communities.

MATERIAL AND METHODS

Loggerhead turtles (N=10 individuals) were sampled around Filicudi Island in the Southern Tyrrhenian Sea (Aeolian Archipelago, Sicily, Italy) in May/June 2016 while swimming at sea. Turtles were captured and measured in terms of curved carapace length (CCL notch-tip), according to Bolten (1999), and their health status was outlined according to ISPRA–MATTM (2013).

The sea turtles epibionts were removed by scraping the posterior part of the carapace by means of a small scalpel, then preserved in polyethylene containers, with 4% formalin and conserved at 4°C. Then in laboratory samples were washed and crustaceans clinging to algae were collected, identified and counted.

RESULT AND DISCUSSIONS

Seven loggerhead turtles were classified as small size (CCL ≤ 50 cm) and three as large size

(CCL > 50 cm). Only one *C. caretta* individual was found dead.

A total of 1120 individuals and 6 species of crustaceans were collected, belonging to amphipods (1002 individuals; 3 species), cirripeds (60; 1), tanaids (57; 1) and isopods (1; 1); they were found at different phases of their life cycle.

The number of species is quite similar among turtles whereas the abundance of species per hosts ranges from 7 to 379.

Six species of crustaceans were found as epibionts. The most abundant colonizer is the free-living amphipods *Caprella andreae* Mayer, 1890 (n= 585 individuals) and *Protohyale grimaldii* (Chevreux, 1891) (n= 416). These species, commonly known to live clinging to floating objects in the high seas, are known as the most frequent amphipods occurring as *C. caretta* epibionts (Kitsos et al., 2005; Sezgin et al., 2009; Zakhama-Sraieb et al., 2010; Casale et al., 2012; Domenech et al., 2014). They were reported by Domenech et al. (2014) as chelonophilic facultative commensals, being able to live as both commensals and free living forms. *P. grimaldii* has been found on flotsam, showing nevertheless a strong association with loggerhead turtles throughout its geographic range (Zakhama-Sraieb et al., 2010). *C. andreae* is a 'rafter' species adapted to live on floating objects (Domenech et al., 2014). However, a recent study suggests that this species prefers to settle on turtles instead of floating objects probably because carapaces provide better conditions for survival (Cabezas et al., 2013).

Other abundant species were the cirriped *Lepas (Anatifa) hillii* (Leach, 1818) (n= 60) and the tanaid *Hexapleomera robusta* (Moore, 1894) (n= 57), found with high abundance only on one specimen of *C. caretta*. The former species is cosmopolite and very abundant as turtles epibiont in the Western and Central Mediterranean Sea (Badillo, 2007; Domenech et al., 2014). According to Badillo (2007) and Domenech et al. (2014), *L. (Anatifa) hillii* can be considered a non-specific species that can colonize both inert or live substrates, and classified as facultative commensal of marine turtles (Frick and Pfaller, 2013). *Hexapleomera robusta* also shows a strong association with marine turtles (Zakhama-Sraieb et al., 2010; Blazewicz-Paszkowycz et al., 2012), although the species is able to live on rock crevices, algae and other solid substrates, including sea mammals, as manatees (Morales-Vela et al.,

2008). The species is considered a chelonophilic facultative commensal by Domenech et al. (2014) and an obligate epibiont of marine turtles by Frick & Pfaller (2013).

The isopod *Idotea metallica* Bosc, 1802 (n= 1), firstly reported as *C. caretta* epibiont by Domenech et al. (2014), was also found in the present study. The amphipod *Isaea montagui* H. Milne Edwards, 1830 (n= 1) is a new record as epibiont of the loggerhead turtle, being known to establish commensal relationship with the spider crab *Maja squinado* (Herbst, 1788). The species gets food from the host as diet debris or faeces (Parapar et al., 1997). Moreover, a recent study (Vader & Tendeberg, 2015) includes this species among the amphipods living directly on the surface of their crustacean hosts, characterised by prehensile pereopods, clearly adapted to the frequent grooming of the host. Hence, we can suppose that the species can establish a similar relationship with the loggerhead turtles.

Further investigations on the crustacean epibionts associated to the loggerhead turtle carapace may be useful to deepen possible factors affecting epibiont recruitment and biodiversity, such as geographic or ecological factors.

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A novel look at the higher classification of Siphonoecetini Just, 1983 (Amphipoda Ischyroceridae)

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ABSTRACT Two new tribes, Ericthonini and Cerapodini, are separated from the Siphonoecetini. The last mentioned is divided into subtribes Siphonoecetina, Bubocoriphina and Caribboecetina.

KEY WORDS Tribes; Siphonoecetini; Ericthonini; Caribboecetini.

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Several amphipod groups utilise extraneous objects (shells, wood, algae) for protective domiciles, but none with a more complex behaviour than that of the siphonoecetids. The aim of this paper is to re-appraise the classification of the siphonoecetids, initially placed in the Siphonoecetinae Just, 1983 and subsequently reduced to Tribe Siphonoecetini in the Ischyrocerinae (Ischyroceridae). At the start of these studies (Just, 1983) the siphonoecetids comprised 16 species in 3 genera. Thirtyfour years later the tally is 62 named species and 19 genera.

The principal papers on which this study is based are Just (1988), Lowry & Berents (1996), and Myers & Lowry (2003). The last mentioned paper placed all genera of the *Siphonoecetes*-clade, its sister group the *Cerapus*-clade and a basic *Ericthonius*-clade in the new tribe Siphonoecetini as sister group to the Ischyrocerini. The *Siphonoecetes*-clade is by far the largest in Myer & Lowry's concept of the Siphonoecetini. I agree with those authors in grouping together those three clades in a sister group position to the only other tribe, Ischyrocerini, in the subfamily Ischyrocerinae, but significant differences among the three clades are blurred and remain cryptic when

all the genera are placed together in the Siphonoecetini.

The study concludes that the three clades currently in the Siphonoecetini require separate tribe status as Ericthonini, Cerapodini and Siphonoecetini as shown in figure 1 and illustrated in figure 2. Further changes to the classification within the new restricted concept of the Siphonoecetini have been adopted during the study with the establishment of three new subtribes: Siphonoecetina, Bubocorophiina and Caribboecetina, and upgrade of subgenera to genus status. These will be shown and discussed.

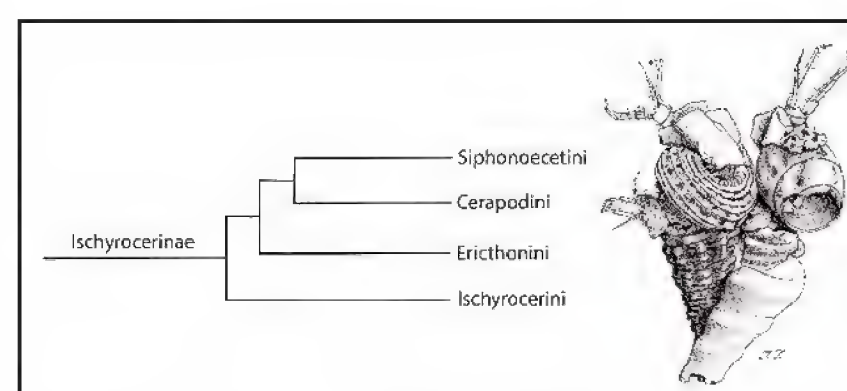


Figure 1. Relationships among the proposed tribes in the subfamily Ischyrocerinae, (an example of abode aggregation in the Siphonoecetini is shown).

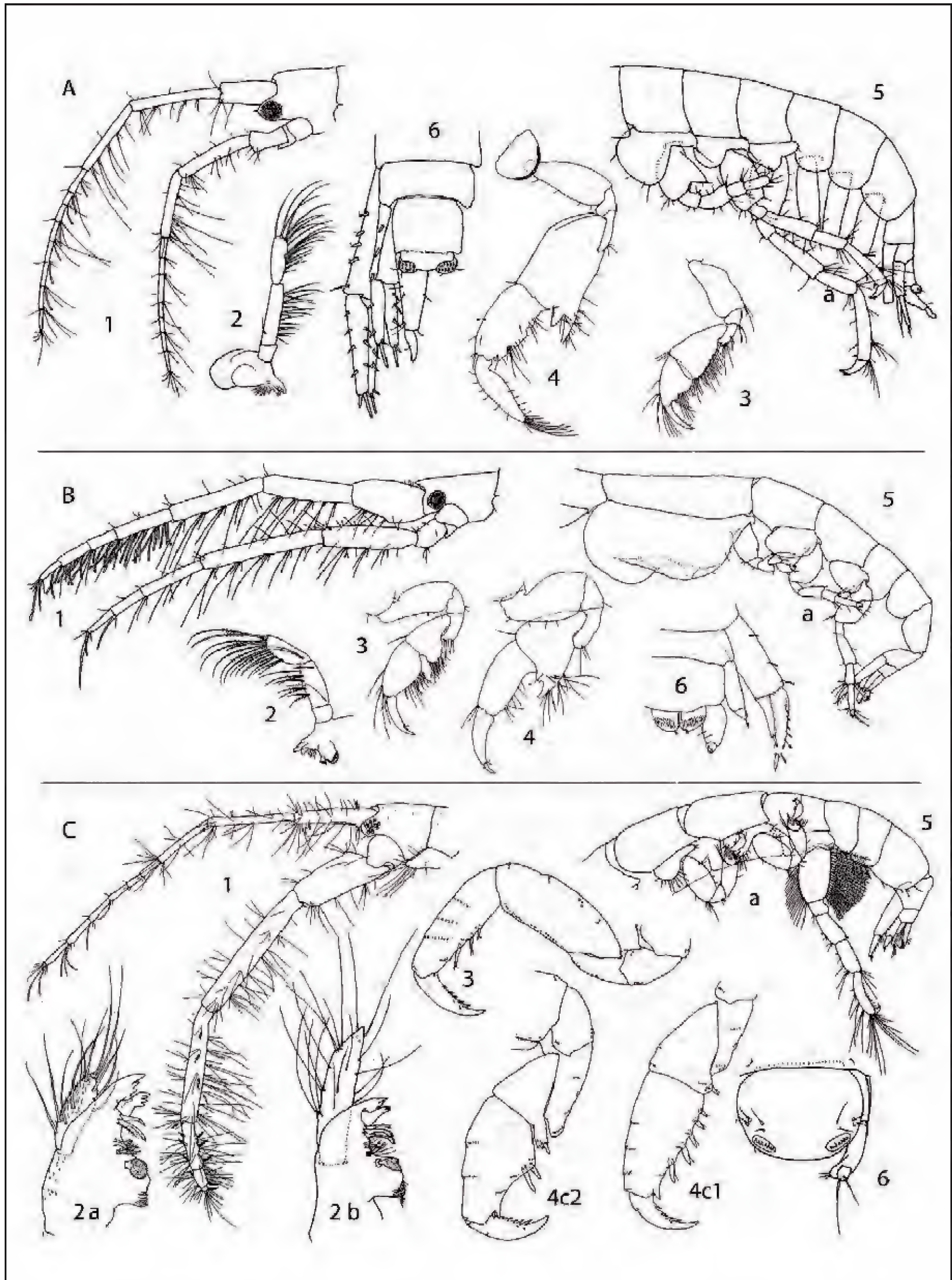


Figure 2. Key characters separating A) Erichthonini, B) Cerapodini and C) Siphonoecetini. 1: antennae; 2: mandible; 3: gnathopod 1; 4: gnathopod 2 (c1 simple and c2 subchelate forms); 5a: pereonite 5 with coxa; 5b: pereopods 5–7; 6: telson with uropods. A2 from Krapp-Schickel (2013); B2 from Lowry & Berents (1996); B6 from Lowry & Berents (2005).

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When to split, when not to split? The Ampeliscidae and Phoxocephalidae as case studies

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ABSTRACT

Due to recent comments on the validity of genera, a discussion on this problem is provided using the amphipod families Ampeliscidae and Phoxocephalidae as case studies. With a view to providing cohesiveness of designation in the amphipod environment.

KEY WORDS

Genus; Phylogenetics; Ampeliscidae; Phoxocephalidae.

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In a recent thread on the discussion list Taxacom, it was commented that genera should not be erected without molecular analysis. The impetus behind this research is to discuss the validity of this comment and to promote discussion for uniformity across different amphipod families. To try and understand these questions the families Ampeliscidae and Phoxocephalidae are used as case studies.

Both the Ampeliscidae and the Phoxocephalidae are strongly species diverse families, which often live in similar habitats (benthic, sediment dwellers). These families have relatively similar numbers of species, the majority of which are valid species (Phoxocephalidae - 369 species and Ampeliscidae with 301 species) with the number of species growing regularly. However, the main difference lies in the number of genera with the Phoxocephalidae

consisting of 79 genera and the Ampeliscidae with only 4.

The questions that need to be asked are that 1) is something happening or has happened evolutionarily to cause more distinct changes at a higher level in the Phoxocephalidae compared to the Ampeliscidae, 2) is something happening biogeographically, with differing feeding lifestyles, 3) are the families, in fact, similarly diverse but with differing levels of undertaken research? 4) if molecular studies need to be carried out to determine genus level status, does every single amphipod (or in fact all living classified) genus need to be reviewed and analysed using molecular methods, and which ones need to be used? And 5) does it even actually matter?

These questions will be discussed with discussions on morphological and molecular methods and the concept of what a genus is.

Response of intertidal amphipod communities to physico-chemical parameters in the Persian Gulf

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ABSTRACT

The relationship between amphipod community and environmental factors was studied on a spatial scale along the Persian Gulf. Seven stations and four environmental factors were considered (seawater temperature, salinity, dissolved oxygen and pH). Ten amphipod species were recorded during this study. Based on the quantitative results *Melita persia* Momtazi, Sari et Maghsoudlou, 2014 and *Parhyale darvishi* Momtazi et Maghsoudlou., 2016 were the most abundant species in the studied area and the highest diversity was seen in the north western part of Qeshm island. Results of Canonical Correspondence Analysis (CCA) showed that salinity is an effective factor among the amphipod assemblages in the Persian Gulf.

KEY WORDS

Melita persia; *Parhyale darvishi*; Persian Gulf; salinity.

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INTRODUCTION

The Persian Gulf is a semi-enclosed sea situated in the subtropical high-pressure-zone and thus characterized by low precipitation and high aridity, resulting in evaporation rates above 2000 mm yr⁻¹ (Barth & Khan, 2008).

The knowledge of the amphipod fauna in the Persian Gulf has been improved in recent years (Myers & Nithyanandan, 2016, Momtazi & Maghsoudlou, 2016, Layeghi & Momtazi, 2015, Momtazi et al., 2014). In the present contribution the community structure of intertidal amphipods was studied in the Persian Gulf and the relationship between abiotic factors and amphipod distribution was discussed.

MATERIAL AND METHODS

Quantitative sampling was done in one transect

by randomly 0.5×0.5 m² quadrat in 7 stations (Fig. 1). Four abiotic parameters were measured in situ (water temperature, pH, dissolved oxygen and salinity). Dissolved oxygen, pH and temperature were measured by Hach HQ11d and salinity by Cond 315i. The relationships between environmental

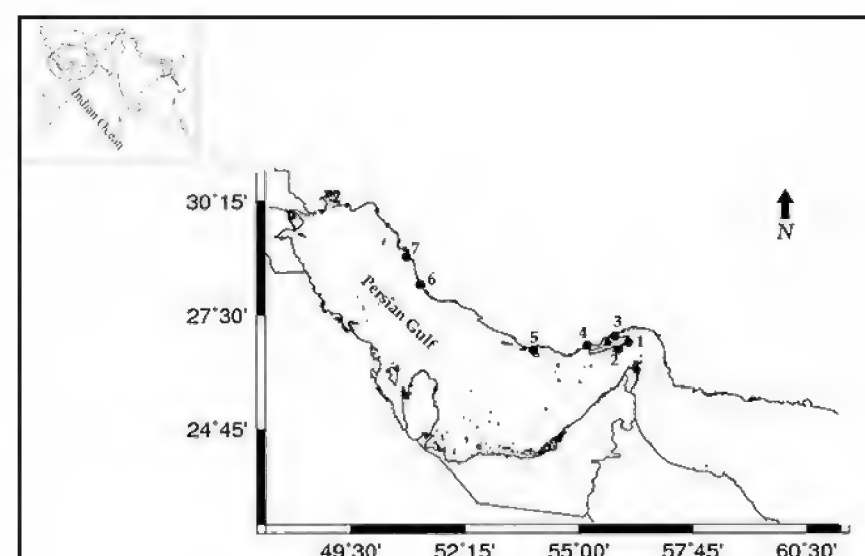


Figure 1. Sampling stations in the present study.

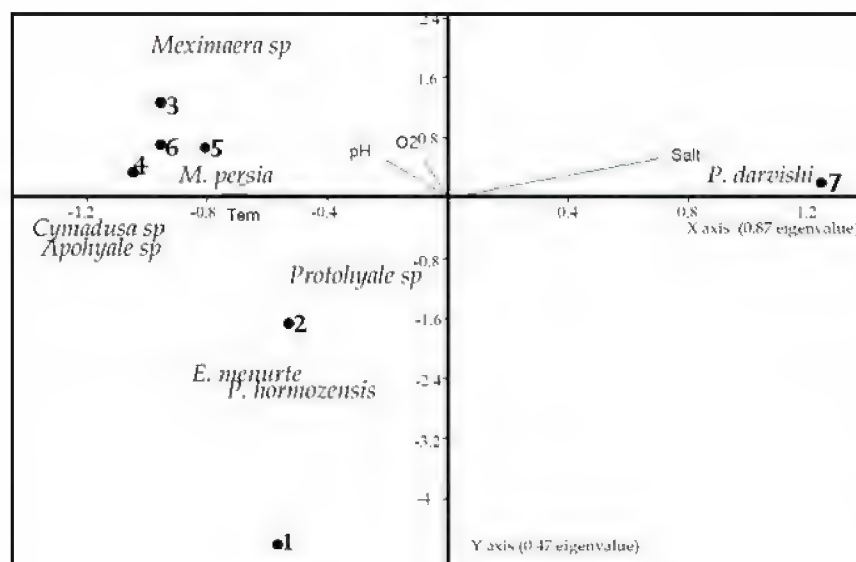


Figure 2. The relationship between environmental factors and amphipod assemblages based on CCA analysis.

measures and amphipod assemblages were studied by Canonical Correspondence Analysis (CCA) using PAST software.

RESULTS AND CONCLUSIONS

The results showed the presence of ten species including: *Ampithoe* sp., *Apohyale* sp., *Cymadusa* sp., *Elasmopus menurte* J.L. Barnard, 1974, *Latigammaropsis pseudojassa* Myers et Nithyanandan, 2016, *Melita persia* Momtazi, Sari et Maghsoudlou, 2014, *Meximaera* sp., *Parhyale darvishi* Momtazi et Maghsoudlou, 2016, *Protohyale* sp. and *Pseudaeiginella hormozensis* Momtazi et Sari, 2013.

Results of multivariate analysis (Fig. 2) showed that the salt concentration is a restrictive factor in the Persian Gulf and except of *Parhyale darvishi*, other species have a negative correlation with salinity. The highest value of Shannon index was calculated for Qeshm Island (Station 1) in the strait of Hormuz at north eastern of the Persian Gulf. The Strait of Hormuz is characterized by multicurrents and less salinity values. The station 7 (Bushehr port) in north western part of the Persian Gulf was marked with the highest value of salinity 43psu and the presence of *P. darvishi*. Among the other abiotic

factors, temperature had also a significant effect in amphipod community. The presence of *Melita persia* as an abundant species is correlated with temperature. The results support the hypothesis that salinity has effect on the amphipod community (Grosse et al., 1987). The two most abundant species *M. persia* and *P. darvishi* in the region seem to vary according with the salinity. When this variable was low (In station 7, Bushehr), *M. persia* was more abundant, but by increasing the salinity, *P. darvishi* substitute *M. persia*.

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Contribution to the knowledge of Iranian southern coast amphipods

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ABSTRACT

The knowledge on amphipods in the intertidal zone of Iranian coasts of Persian Gulf and the Gulf of Oman was improved during last five years. Sampling was done at 55 stations and 32 species of 14 family were recorded. The new described species (9 species) in this study composed 28% of the total amphipod fauna and eleven species (34%) are waiting to be described. The environmental character and geological history are the main factors for a high endemism and the differences between the southern and the northern coast of the Persian Gulf and the differences between the Gulf of Oman and the Persian Gulf.

KEY WORDS

Amphipoda; Persian Gulf; Gulf of Oman.

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INTRODUCTION

There are a few studies on Amphipoda of the Persian Gulf (PG) and the Gulf of Oman (GO) (Myers, 1981; Salman, 1985, 1986, 1998; Barnard & Thomas, 1984; Myers & Nithyanandan, 2016). In the present contribution a checklist of the amphipods along with their distributional pattern for the region is provided.

MATERIAL AND METHODS

Sampling was done during 2011-2016 at 50 stations along the intertidal zone of southern Iranian coasts (Fig. 1). Material was deposited in the Iranian National Institute for Oceanography Collection (INIOC) and the Zoological Museum, University of Tehran (ZUTC).

RESULTS

Thirty two species of 14 families were recorded during this study (Table 1). By adding previously described species, the amphipod diversity in the Persian Gulf and the Gulf of Oman reached 43 species. The fauna is composed of 71% endemic, 19% Indian Ocean and 10% cosmopolitan elements. A comparison with studies from Kuwait shows that there is only 21% similarity between the southern and the northern coasts of PG. There was 30% similarity between species composition in the Persian Gulf and the Gulf of Oman.

The different geology as well as environmental conditions of the PG and GO region were considered as possible factors for the high endemism. However, completion of the information gap in the future for the Western Indian Ocean amphipods may modify our hypothesis.

Family	Species	PG	GO	Distribution
Ampeliscidae	<i>Ampelisca cyclops</i>	-	+	Indian ocean
	<i>Byblis lepta</i>	-	+	Indian ocean
Ampithoidae	<i>Cymadusa</i> sp. [°]	-	+	endemic
	<i>Cymadusa setosa</i>	+	+	cosmopolitan
	<i>Cymadusa filosa</i>	+	+	cosmopolitan
	<i>Ampithoe</i> sp. [°]	+	+	endemic
	<i>Ampithoe qeshm</i> [*]	+	-	endemic
	<i>Plumithoe hirsuta</i>	-	+	Indian ocean
Aoridae	<i>Grandidierella</i> sp. [°]	-	+	endemic
	<i>Bemlos acuticoxa</i>	+	-	endemic
Caprellidae	<i>Metaprotella macoranicus</i> [*]	+	-	endemic
	<i>Monoliropus kazemii</i> [*]	+	-	endemic
	<i>Pseudocaprellina pambanensis</i>	-	+	Indian ocean
	<i>Pseudaeginella hormozensis</i> [*]	+	-	endemic
	<i>Caprella</i> sp. [°]	-	+	endemic
Leucothoidae	<i>Leucothoe</i> sp. [°]	-	+	endemic
Lysianassidae	<i>Shoemakerella</i> sp. [°]	-	+	endemic
Hyalidae	<i>Parhyale darvishi</i> [*]	+	+	endemic
	<i>Apohyale</i> sp. [°]	+	+	endemic
	<i>Protohyale</i> sp. [°]	+	+	endemic
Maeridae	<i>Glossomaera octodens</i>	+	+	Indian ocean
	<i>Elasmopus alkhiranensis</i> [*]	+	-	endemic
	<i>Elasmopus menurte</i>	+	-	Indian ocean
	<i>Meximaera</i> sp. [°]	+	-	endemic
Melitidae	<i>Melita persia</i> [*]	+	+	endemic
Photidae	<i>Latigammaropsis pseudojassa</i>	+	-	endemic
Podoceridae	<i>Podocerus mamlahensis</i>	+	-	endemic
	<i>Podocerus</i> sp. [°]	-	+	endemic
Pontogeneiidae	<i>Tethygeneia</i> sp. [°]	+	-	endemic
Talitridae	<i>Talorchestia qeshm</i> [*]	+	-	endemic
	<i>Persianorchestia nirvana</i> [*]	-	+	endemic
Urothoidae	<i>Urothoe grimaldii</i>	-	+	cosmopolitan

Table 1: Recorded amphipod species in the studied area.
° is specified the new species that is under publishing; * is specified the new described species in the region.

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Biodiversity of subtidal amphipod assemblages from Chabahar Bay, Makran Sea, Iran

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ABSTRACT

Subtidal amphipod assemblages were studied in Chabahar Bay. Sampling was done in 16 stations by Ekman grab 0.25 m² in October 2016. The material belonged to 21 species. The higher Shannon index was calculated for the entrance of the Bay where currents were stronger. *Ampelisca cyclops* Walker, 1904 (Ampeliscidae) was the most abundant species in the studied area. A nMDS analysis showed a separation between amphipod assemblages of the stations at the entrance of the bay compared to stations inside the bay.

KEY WORDS

Subtidal amphipods; Chabahar Bay; *Ampelisca cyclops*.

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INTRODUCTION

The Chabahar Bay is deep and Ω-shaped. It is part of the Makran area in the southeast of Iran (Oman Sea, north-western part of Indian Ocean). It has a 13.5 km wide entrance and 17 km length in south-north direction. The amphipod fauna of the Chabahar Bay generally had been ignored in macrobenthos studies particularly in the subtidal region.

The biodiversity of subtidal macrobenthos amphipods were evaluated in the present study.

MATERIAL AND METHODS

Quantitative sampling was done with three replicates by a 0.25 m² Ekman grab, in 16 stations in November 2016 (Fig. 1). The contents of each grab were sieved with 0.5 mm mesh size and the material was fixed in 75% ethanol. Dissections were made in glycerol. Diversity indices and mul-

tivariate analyses were carried out by the PAST software.

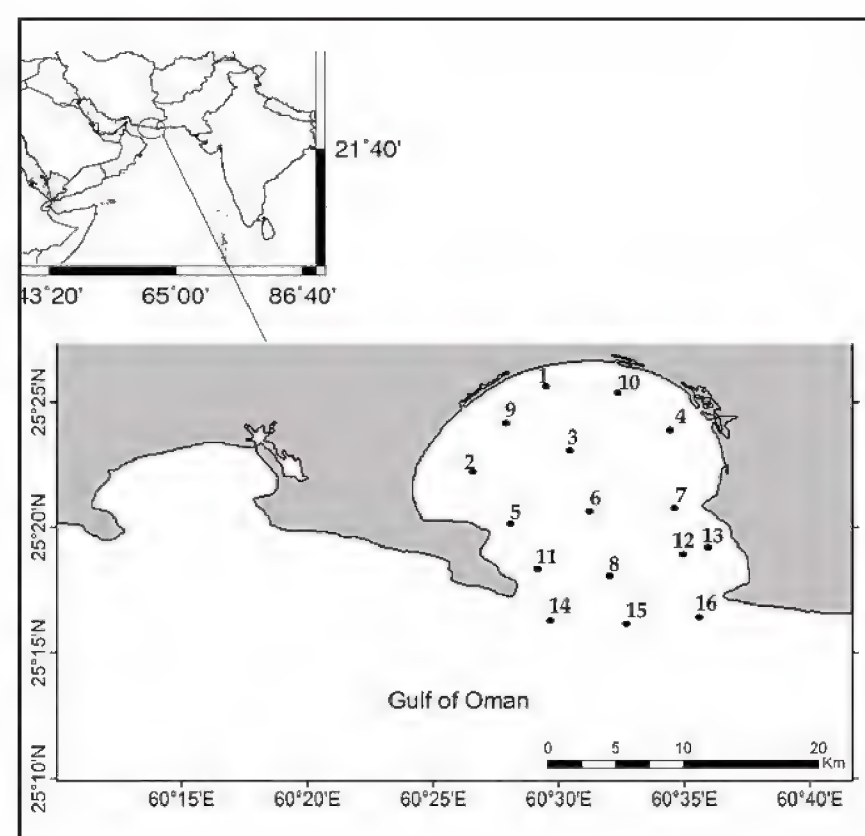


Figure 1. The sampling network in the Chabahar Bay.

RESULTS AND CONCLUSIONS

The collected material belongs to 21 species. *Ampelisca cyclops* was dominant and the most abundant. The highest diversity was calculated for the entrance of bay (station 15, Table 1). West head-land stations also show high diversity indices.

The results of Non-metric multidimensional scaling (nMDS, Fig. 2) show a similarity between stations at the entrance of bay (marked by elliptic in figure). However, a relation between other stations

could not be described based on available data and may be affected by other factors.

The patterns of the water currents in the bay seem to be as the main factor for the amphipod community structure and the higher biodiversity at the entrance of bay. The combination of wind-driven and tidal currents generate a self-flushing mechanism in the bay that tend to carry suspended material outside of the bay (Hassannezhad et al., 2011), and increase the amount of dissolve oxygen in the ecosystem.

station	S1	S2	S3	S4	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16
Species richness	4	2	1	1	3	3	4	3	2	8	2	3	4	9	3
Individuals	19	7	1	6	3	4	5	6	32	20	1	5	16	29	10
Shannon_H	0.73	0.41	0	0	1.09	1.04	1.33	1.01	0.13	1.87	0	0.95	0.68	2	0.64
Simpson_1-D	0.36	0.24	0	0	0.66	0.6	0.72	0.61	0.06	0.82	0	0.56	0.32	0.83	0.34

Table 1. Biodiversity indicescalculated for each station.

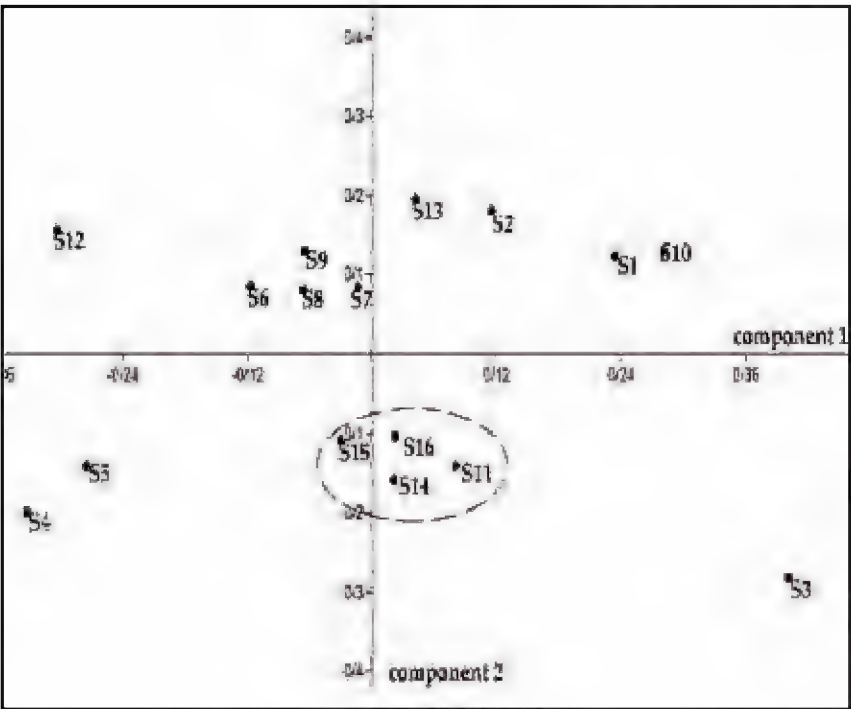


Figure 2. The results of the nMDS analysis.

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Origin, dispersals and diversification dynamics of Epimeriidae and Iphimediidae (Amphipoda Crustacea) from the Antarctic shelf

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ABSTRACT

The origin, potential dispersals in/out of the Southern Ocean and *in situ* diversification patterns of Antarctic epimeriids (genus *Epimeria* Costa in Hope, 1851) and iphimediids were inferred based on time-calibrated phylogenies of Antarctic and non-Antarctic representatives of the two families.

KEY WORDS

Amphipoda; southern Ocean; historical biogeography; phylogeny; divergence times.

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INTRODUCTION

The physical isolation of the Antarctic shelf and extreme life conditions contribute to its high degree of endemism. The Antarctic shelf fauna would, however, be composed of Gondwanan descendants, but also of more recent colonizers.

The peculiar climatic history of this region might have provided environmental prerequisites to the radiation of some lineages, some of which might afterwards colonize the other ocean’s shelves. Amphipods from the families Epimeriidae and Iphimediidae are cosmopolitan, but well-represented on the Antarctic shelf.

Antarctic epimeriids (represented herein by the genus *Epimeria* Costa in Hope, 1851) are composed of strictly endemic and presumably more generalist species, while many Antarctic iphimediids appear to be food specialists, with some of them distributed on both sides of the Polar Front.

MATERIAL AND METHOD

By reconstructing time-calibrated phylogenies based on mitochondrial (COI) and nuclear (28S and H3) markers and including representatives from the Southern Ocean and other world’s oceans, this study aims to investigate, for each of these two families, the origin of the Antarctic component, their propensity towards dispersals in/out of the shelf and the *in situ* diversification patterns. A comparison of observed biogeographic patterns for the two families will give insights into the influence of historical environmental factors on the evolutionary history of organisms with contrasting life history traits.

RESULTS AND CONCLUSIONS

In both phylogenetic reconstructions, all Antarctic and sub-Antarctic (for iphimediids) species

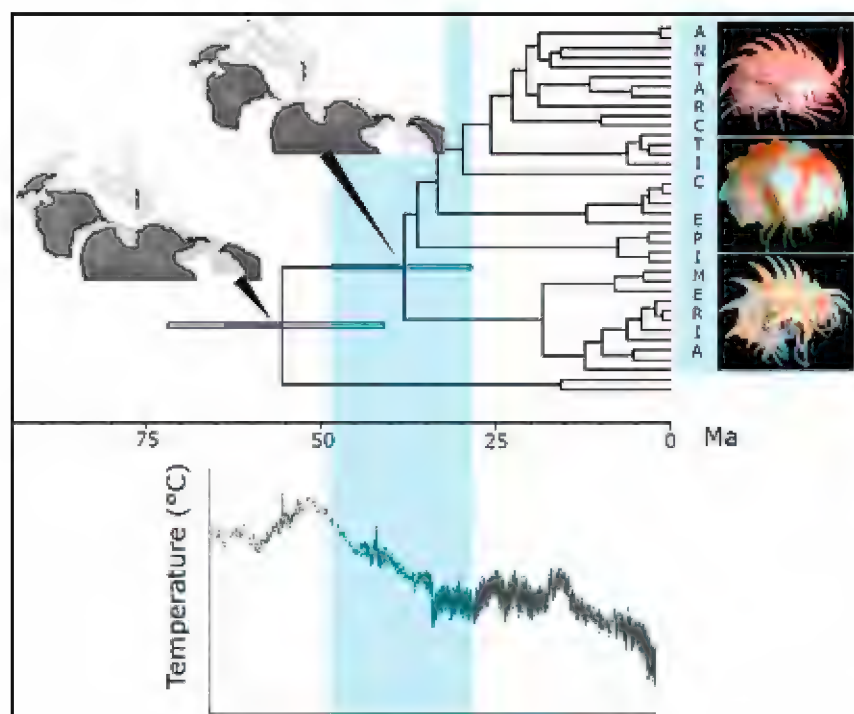


Figure 1. Graphical abstract of the biogeographical study of Antarctic *Epimeria*.

formed a clade, sister to a non-Antarctic clade. While the precise timing of origin for the (sub-)Antarctic components could not be inferred in both cases, the (sub-)Antarctic lineages likely arose from late Gondwanan ancestors and hence, did not colonize the Antarctic region after the continent broke apart from the other fragments of Gondwanaland.

Moreover, the initial diversification of these two clades occurred during the progressive transition to an Icehouse climate and would therefore be related to cold-waters (Fig. 1 for *Epimeria*).

A diversification burst, possibly related to glacial cycles, might have occurred within one (or two)

iphimediid subclade(s) after the mid-Miocene Climate Transition. While the Antarctic *Epimeria* lineage appears “locked in the Icehouse”, iphimediids historically dispersed at least once from the high Antarctic region to sub-Antarctic islands, after the geographical isolation of Antarctica. However, based on the present taxon sampling, they do not appear to have dispersed further north than sub-Antarctic regions at any point of their evolutionary history.

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Implantable pH-sensitive optical microsensors for *in vivo* stress assessment of amphipods

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ABSTRACT

In this study we developed a technique for application of fluorescent microsensors for *in vivo* monitoring of pH in adult amphipods. The microsensors demonstrated higher sensitivity to physiological changes in amphipods exposed to hypoxic conditions than common biochemical stress markers.

KEY WORDS

Amphipoda; Baikal; microencapsulated biomarkers; *Eulimnogammarus verrucosus*.

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One of the limits of methods in modern eco-physiology is restricted possibility of repeated measurements of internal physiological characteristics for prolonged assessment of the organism stress reactions under certain conditions. It is also the case for most amphipod species as repeated sampling of amphipod hemolymph or tissues for biochemical and molecular analysis is usually practically impossible. This limitation does not allow following individual-specific physiological reactions and also significantly increases the number of animals required for each experiment.

A promising solution to this problem is application of implantable optical microsensors for direct monitoring of physiological characteristics of amphipod hemolymph in the very the same individuals during the whole experiment. A type of microsensors, which can be applicable for this purpose, is microencapsulated fluorescent probes. There is a wide variety of commercially available fluorescent molecular probes sensitive to such parameters as pH, different ions and metabolites (Johnson &

Spence, 2010), while encapsulation of the dyes to semipermeable shells allows reducing their possible toxicity and concentrating the probe at one point.

In the current study we applied *in vivo* pH-responsive fluorescent microsensors to evaluate their sensitivity to physiological changes in hemolymph of amphipods under hypoxic conditions and compare it with sensitivity of common biochemical stress markers. The microsensors (prepared as described by Kreft et al., 2007; Sadovoy et al., 2012) were introduced into circulatory system of amphipods and visualized by a fluorescent microscope connected to a spectrometer to analyze the optical signal from the sensor. Endemic species of amphipods *Eulimnogammarus verrucosus* (Gerstfeldt, 1858), which is abundant in littoral zone of Lake Baikal, was chosen as the object of research. Lake Baikal is now subjected to massive proliferation of algae and macrophytes in some coastal areas (Timoshkin et al., 2016) that can lead to temporary changes in gas regime of parts of the lake littoral zone such as increase in carbon dioxide concentra-

tion and reduction in oxygen content (both lead to hypoxia and, potentially, shift in hemolymph pH). Activities of antioxidant enzymes (catalase, peroxidase and glutathione S-transferase) as well as lactate content were evaluated as sensitive biochemical markers of stress response for amphipods (Axenov-Gribanov et al., 2016).

Amphipods were caught by kick-sampling in littoral of Lake Baikal and acclimated in aerated aquaria with temperature $\sim 6^\circ\text{C}$ (average annual temperature in littoral zone; was controlled during all experiments). To make sure injections of the fluorescent microsensors do not lead to significant changes in the organism, we evaluated hemolymph pH and the chosen biochemical parameters 6 h after injections of the microsensors. No changes in hemolymph pH, enzyme activities or lactate content were identified after this period. Hemolymph pH measured *in vivo* by microsensors (median ~ 8.2) at these conditions also was in agreement with pH measurements with a microelectrode in extracted hemolymph.

At first, we exposed *E. verrucosus* to elevated carbon dioxide level (55–70 mg/l) during 2 h. Activities of antioxidant enzymes and lactate content did not change significantly under these conditions, but variation in lactate level increased almost twice. On the contrary, median hemolymph pH (monitored *in vivo*) decreased from ~ 8.2 to ~ 7.7 and the difference was statistically significant.

Then, amphipods were subjected to exposure without aeration during 18 h (carbon dioxide increased from 1–2 to 4–6 mg/l, oxygen changed from 11–12 to 8–10 mg/l). Again, no changes in antioxidant enzymes' activities were observed, while median of lactate content significantly rose from 0.1 to 0.2 $\mu\text{M/g}$. Median hemolymph pH in these conditions also changed from control level to ~ 7.7 .

Thus, our results demonstrate that sensitivity of optical microsensors to physiological changes induced by some stressors can be equal to or even exceed sensitivity of common biochemical stress markers. Moreover, the time required for hemolymph pH analysis with the proposed technique is much less than the time of spectrophotometric bio-

chemical measurements, while the cost is comparable. Since many species of amphipods are at least partially transparent to visualize the fluorescent microsensors in their circulatory system, this *in vivo* technique can find a wide range of applications in ecophysiological research on amphipods.

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Population structure of amphipods *Eulimnogammarus verrucosus* (Gerstfeldt, 1858) and *E. cyaneus* (Dybowsky, 1874) endemic to Lake Baikal

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ABSTRACT

In this study we examined population structure of two congener species of amphipods *Eulimnogammarus verrucosus* (Gerstfeldt, 1858) and *E. cyaneus* (Dybowsky, 1874) endemic to Lake Baikal using a fragment of *cytochrome c oxidase* gene. Despite both *E. verrucosus* and *E. cyaneus* inhabit upper littoral zone of the lake, a dramatic difference in geographical variability of the fragment was identified between these species.

KEY WORDS

Amphipoda; Baikal; COI; COX1; *Eulimnogammarus*.

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Lake Baikal is inhabited by an extraordinarily diverse fauna with a high degree of endemism. The lake is a hotspot of species diversity of amphipods (Amphipoda, Crustacea), a major benthic taxon. We raised the question whether amphipods *Eulimnogammarus verrucosus* (Gerstfeldt, 1858) and *E. cyaneus* (Dybowsky, 1874), two highly abundant littoral species, form homogenous populations in the lake or whether separate populations can be distinguished. *Eulimnogammarus cyaneus* is a thermotolerant species and inhabits depths 0–1 m, while *E. verrucosus* is more thermosensitive and can be found at higher depths down to 6 m (Bazikalova, 1945). Thus, we hypothesized that *E. cyaneus* may be subjected to higher separation by geographical barriers than *E. verrucosus*.

We investigated the population structure of these two species on the basis of a gene segment (length

>600 bp) of the *cytochrome c oxidase subunit I* (COI). We sequenced this gene fragment in animals sampled at 18 sites (5–15 individuals per site) around Lake Baikal, including an island Bolshoy Ushkaniy located nearby the east coast of the lake.

The phylogenetic networks based on the sequence alignments demonstrate dramatically different intraspecific variabilities of the COI fragment between the two species: genetic distances within *E. cyaneus* do not exceed 3% (of the fragment length), while maximal genetic distances within *E. verrucosus* can reach 14%. Moreover, sequences of *E. verrucosus* cluster together to 3 clades by geographical location of the sampling site, and each clade has internal genetic distances of no more than 3%. The clades are separated by such geographical barriers as the Angara river outflow; probably, the Selenga river delta; and, possibly, a not identified

geographical barrier at the eastern shore of northern Baikal. The population of Bolshoy Ushkaniy island appears to be more closely related to amphipods at the western shore than to animals from closely located eastern shore. In case of *E. cyaneus* no separation of sequences by geographical location was found. Three identified clades of *E. verrucosus* may represent both isolated populations of this species or different cryptic species.

Thereby, our results do not support the original hypothesis of this study, and show that *E. cyaneus* is much more homogeneous than *E. verrucosus*. These data also support the hypothesis about accelerated evolution rate in amphipods of Lake Baikal (Romanova et al., 2016; Naumenko et al., 2017).

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Can adaptation to habitats with different salinity regimes modulate the energy metabolism, cellular stress responses and thermal tolerance in amphipod *Gammarus lacustris* Sars, 1863?

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ABSTRACT

We investigated the influence of different salinity regimes on thermotolerance, energy metabolism and cellular stress defense mechanisms in amphipods *Gammarus lacustris* Sars, 1863 from two populations. Amphipods from a freshwater population were more sensitive to the thermal challenge, showing higher mortality during acute and gradual temperature change, compared to their counterparts from a saline lake. A more thermotolerant population from a saline lake had high activity of antioxidant enzymes. The energy limitations of the freshwater population (indicated by low baseline glucose levels, downward shift of the critical temperature of aerobic metabolism and inability to maintain steady-state ATP levels during warming) was observed, possibly reflecting a trade-off between the energy demands for osmoregulation under the hypo-osmotic condition of a freshwater environment and protection against temperature stress.

KEY WORDS

Thermal tolerance; salinity; *Gammarus lacustris*; adaptation; Amphipoda.

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Temperature and salinity are important abiotic factors affecting survival and performance of aquatic invertebrates and setting limits to their geographical distribution (Pörtner, Farrell, 2008). The temperature has a significant effect on the ectothermic animals such as amphipods, since the rate of physiological and biochemical reactions in organisms directly depend on the environment temperature. The thermal fluctuations are often associated with changes in other abiotic factors, for example, such as salinity. Therefore, environmental stress (including salinity and temperature stress) can significantly influence the energy balance of living organisms due to the additional energy required to

restore and maintain homeostasis, which can put a strain on the energy acquisition, transformation and conservation systems (Sokolova et al., 2012).

The aim of our study was to determine whether adaptation to habitats with different salinity regimes modulates the energy metabolism, cellular protective responses to temperature stress and thermal tolerance of amphipod *Gammarus lacustris* Sars, 1863.

We collected amphipods from two different habitats (a freshwater habitat and a saline lake) in Eastern and Western Siberia (Russia). We exposed the amphipods to several thermal scenarios (namely, hypothermia, as well as acute and gradual warming)

and determined their survival, activity of major antioxidant enzymes and parameters of energetic metabolism in order to gain insights into the physiological and cellular mechanisms of temperature-salinity interactions in this ecologically important euryoecious species.

Our study shows that adaptation to different salinity regimes can influence thermotolerance and modulate key characteristics of cellular metabolism and stress responses in Holarctic amphipods *G. lacustris*. Amphipods from the freshwater Irkutsk population were more sensitive to the thermal challenge experiencing higher mortality during acute and gradual warming compared to the amphipods from the saline Lake Shira. Median mortality times (LT50) during acute exposure to 30 °C were significantly higher in amphipods from the saline Lake Shira (LT50 = 22.8 h) compared to their counterparts from a freshwater lake in Irkutsk (LT50 = 7.7 h). A total of 100% mortality during gradual warming was observed at 31 °C in Irkutsk population and at 33 °C in Shira population.

The thermal challenge led to a significant increase in activities of all tested antioxidant enzymes (peroxidase, GST and catalase) in the freshwater population of amphipods. Elevated levels of antioxidant enzymes during the thermal challenge may reflect a temperature-induced increase in generation of reactive oxygen species (ROS) in the less tolerant freshwater population requiring upregulation of the cellular antioxidant capacity to protect the organism against oxidative stress. This is unlikely to indicate a direct thermal damage to the antioxidant enzymes given high tolerance to heating in this population but may rather reflect a decrease in the ROS production at intermediate temperatures. Metabolic responses to thermal challenge notably differed in the freshwater and saltwater populations. The stable levels of ATP during the acute temperature stress in the saltwater amphipods indicates that the compensatory onset of anaerobic ATP production in combination with the aerobically produced ATP is sufficient to prevent ATP depletion. Significantly lower baseline levels of glucose in a freshwater population indicates that it may be energy-limited. Activation of glycogenolysis, and

the onset of anaerobiosis (indicated by lactate accumulation) occurred much later in the freshwater amphipods.

Our data indicate that higher thermal sensitivity of the freshwater population of amphipods is associated with a lower baseline activity of antioxidant enzymes and a decreased ability to maintain energy balance and curb oxidative stress, compared to their saltwater counterparts, during exposure to acute and gradual temperature increase. High sensitivity of the freshwater population to warming was associated with energy limitations, possibly reflecting a trade-off between the energy demands for osmoregulation and protection against the temperature stress. These findings suggest that freshwater populations of amphipods may be more vulnerable to the global climate change than those from saline habitats. On the other hand, brackish waters may serve as potential refuges during the climate change for euryhaline amphipod species such as *G. lacustris*.

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Effects of rising temperatures on expression patterns of genes related to universal stress-response and energy metabolism in Baikal endemic and Holarctic amphipods

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ABSTRACT

This study investigated gene expression of heat shock protein 70 (hsp70) and several key metabolic enzymes in two congener amphipod species endemic to Lake Baikal - *Eulimnogammarus verrucosus* (Gerstfeldt, 1858) and *Eulimnogammarus cyaneus* (Dybowski, 1874), and the Holarctic *Gammarus lacustris* Sars, 1863 - exposed to a temperature increase of 0.8 °C d⁻¹ during a 22-days exposure. Upregulation of hsp70 expression was found in all the studied species at a temperature of 23.6 °C. Gradual temperature increase altered the expression of metabolic genes - oxoglutarate dehydrogenase, hexokinase, sodium-potassium adenosine triphosphatase and atp synthase subunit alpha only in *E. cyaneus* that is endemic to Lake Baikal. Correlation network analysis revealed different strategies of energy supply and stress-response (hsp70 induction) under elevated temperatures in the studied species.

KEY WORDS

Amphipoda; Baikal; gene expression; energy metabolism.

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Temperature is one of the most important environmental factors for aquatic organisms due to its direct effects on all biological processes - from the molecular to the physiological and behavioral hierarchy of organization. Changes in habitat temperature can occur on a wide temporal range - from daily or tidal cycles to large-scale climatic fluctuations. Thus, understanding of thermal ranges and limits of organisms becomes increasingly important in the light of climate change and its observed effects on ecosystems (Pörtner & Gutt, 2016).

The aim of our study was to investigate the expression of several key metabolic genes in response to gradually increasing temperature (0.8 °C per

day) in two Baikal endemic amphipods and one common Holarctic species. The studied species will have to cope with the projected global warming during the next decades (IPCC, 2007). In this context, the thermal tolerance of aquatic organisms can be considered as a crucial biological trait.

For the gradual temperature increase experiment, tanks with amphipods acclimated to 6 °C were placed to the laboratory incubator and water temperature was increased by 0.8 °C per day. The rate of increase intended to resemble natural conditions of the upper littoral zone of Lake Baikal as maximal temperatures can reach about 20 °C in summer (Timoshkin, 2001), whereas in spring/early

summer temperatures of around 5–6 °C prevail in the upper littoral. The experimental temperature increase slightly exceeded temperature changes in the natural habitat (regarding the thermal increments and the maximum temperature) in order to account for a climate warming scenario. Control groups of amphipods were kept at 6 ± 0.8 °C in a laboratory incubator during the entire experimental period.

Expression of heat shock proteins 70 (hsp70) significantly increased in the endemic Baikal species *E. cyaneus* and the Holarctic *G. lacustris* at 23.6 °C (after 22 days of gradual warming exposure), compared to the parallel (time) control. In another Baikal endemic amphipod - *E. verrucosus* - gradual temperature exposure resulted in increased hsp70 expression at 23.6 °C, however, only when compared to the expression level in start control samples. In the Baikal endemic *E. cyaneus*, expression of oxoglutarate dehydrogenase and hexokinase also increased at 23.6 °C. Expression of atp synthase subunit alpha significantly decreased in this species after 16 days of gradual warming (at 18.8, 22 and 23.6 °C, respectively). This could indicate the regulation of energy metabolism in *E. cyaneus* in response to temperature stress, which was not observed in the other two species. Correlation network analysis revealed different strategies of energy

supply and stress-response (hsp70 induction) under increasing temperatures in the studied species.

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The Amphipoda of the Gulf of Cadiz: between cold seeps and coral mounds

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ABSTRACT

The diversity of substrates occurring in the Gulf of Cadiz (SW Iberian Peninsula) lead to the presence of a variety of Amphipod communities that are presented in this work.

KEY WORDS

Mud volcanoes; coral mounds; boxcores; diversity; distribution.

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The Gulf of Cadiz (GoC; see Fig. 1) is an extensive methane seep area in the SW Iberian and Moroccan margins (NE Atlantic). Here, the mud volcanoes (MVs), piercing a thick accretionary prism, are divided into three regions with differentiated geological, geochemical and oceanographic characteristics: El Arraiche field (down to 500 m depth bounded by the Pen Duick Escarpment and Vernadsky Ridge), in the upper slope of the Moroccan margin, influenced by the upper branch of the Mid-Atlantic Water; the Carbonate province (700–1000 m), rich in authigenic carbonates and coral mounds and influenced by the Mediterranean Outflow Water; the Deep field (1300–4000 m), in the lower slope and influenced by the high in nutrients Antarctic Intermediate flow. The Horseshoe field (4000–4500 m) is on the Horseshoe Continental Rise (HCR), along the Southwest Iberian Margin (SWIM) fault, lying on oceanic crust and with hydrothermal influence (Cunha et al., 2013).

The aim of this study was to a) gaining information on the biodiversity and distribution ecology of the Amphipoda in the area; b) assessing differences in faunal composition and diversity between mud volcanoes and coral mounds; and c) assessing spa-

tial distribution patterns of the Amphipoda in the GoC. For this purpose, specimens from a total of 182 samples taken with different sampling methods during several research cruises carried out between 2000 and 2012 were examined. Multivariate analyses were performed on species presence/absence data. Further statistical analyses were carried out on data resulting from quantitative samples (i. e., boxcores).

A total of 3982 specimens belonging to 150 nominal species were identified, with a 13.3% of singletons; a nearly asymptotic global rarefaction curve suggest that the amphipods diversity of the GoC is nearly covered by this study, nevertheless, there is a number of species that remain to be described. The most abundant species was *Valetietta* sp.A, which was found in high numbers at Darwin MV, followed by the phoxocephalid *Harpinia pectinata* and the caprellid *Liropus* aff. *elongatus*. On the other hand, the most ubiquitous species was *Harpinia* aff. *crenulata* which was present in 33 samples, followed by *Oediceroides pilosa*. Quantitative analyses showed that the assemblages in the mud volcanoes were significantly different from those in coral mounds; the species that most contributed to such differences were *Stenothoe* aff.

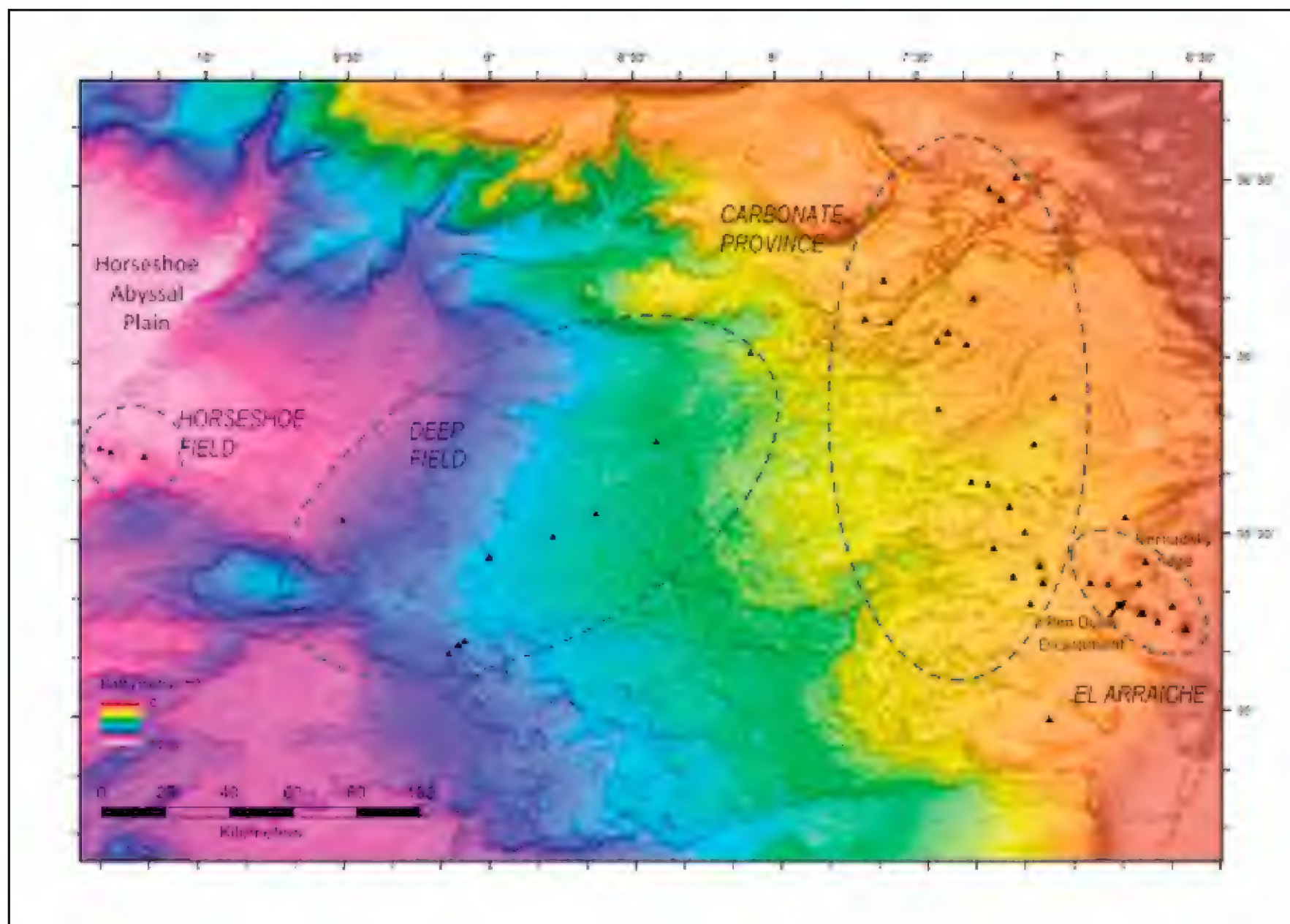


Figure 1. The Gulf of Cadiz, showing the four mud volcano fields. Triangles: md volcanoes. Modified from Cunha et al. (2013). Bathymetry from Zitellini et al. (2009).

marina and *Harpnia* aff. *crenulata*. Although diversity, species richness and abundances were higher in samples taken in mud volcanoes than those in coral mounds, the differences were not statistically significant.

On the other hand, there is a clear, although gradual species turnover from the El Arraiche field to the HCR, with a constant species loss and gain with depth. This allows us to infer that, the species composition of the amphipod communities in the mud volcanoes do not vary in response to the varying geochemical conditions across the various fields, instead, they vary responding to depth.

We can conclude that the species composition of the amphipod communities in the Gulf of Cádiz are influenced by both the type of substrate (coral mounds or mud volcanoes) and depth, while species

richness and abundances do not seem to respond to a particular pattern.

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Reconciling large molecular datasets, bioinformatics and taxonomy: prospects for Amphipoda

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ABSTRACT

Amphipod DNA sequences have been accumulating at a rapid pace during the last decade, generating a large body of data. Here we summarize progress and provide insights into potential usage of such large datasets.

KEY WORDS

Amphipoda; DNA barcoding; BINs; taxonomy.

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INTRODUCTION

In the context of increased human impact on biodiversity resulting, among others, in high extinction rates, there was a need for fast inventories of the living world. The development of molecular technologies responded to these needs and resulted in the accumulation of large volumes of molecular information. In particular, the rise in popularity of DNA barcoding produced a considerable amount of data during the last decade. DNA barcoding is a molecular tool for species identification based on sequencing a short fragment of the mitochondrial cytochrome c oxidase I gene (COI) in animals. Its success relies on the existence of: 1) reference barcode libraries, in which specimens are identified with traditional approaches (i.e., morphological characters) and then sequenced, and 2) the barcode gap, in which intraspecific genetic distances are lower than interspecific distances. The main storage and analytical platform for DNA barcodes, the Barcode of Life Data Systems (BOLD), currently holds over 6 million DNA sequences for various taxono-

mic groups, providing great opportunities for biodiversity studies. Due to the fast pace in generating DNA sequences and the delay in taxonomic identifications, new computer-based tools (e.g., various algorithms), have been introduced in the analytical molecular pipeline. One such tool, the Barcode Index Number (BIN) available in BOLD, produces clusters of barcodes with unique identifiers that can be used as proxies for species.

Here we investigate the progress in accumulating COI sequences for both freshwater and marine amphipods and the utility of large molecular datasets for taxonomy. In particular, we were interested in finding cases of disagreement between molecular (BINs) and morphological (species names) clusters at the global scale and in providing some prospects into future approaches for barcoding.

MATERIAL AND METHODS

BOLD is constantly mining data from GenBank (the largest molecular database) in order to gather

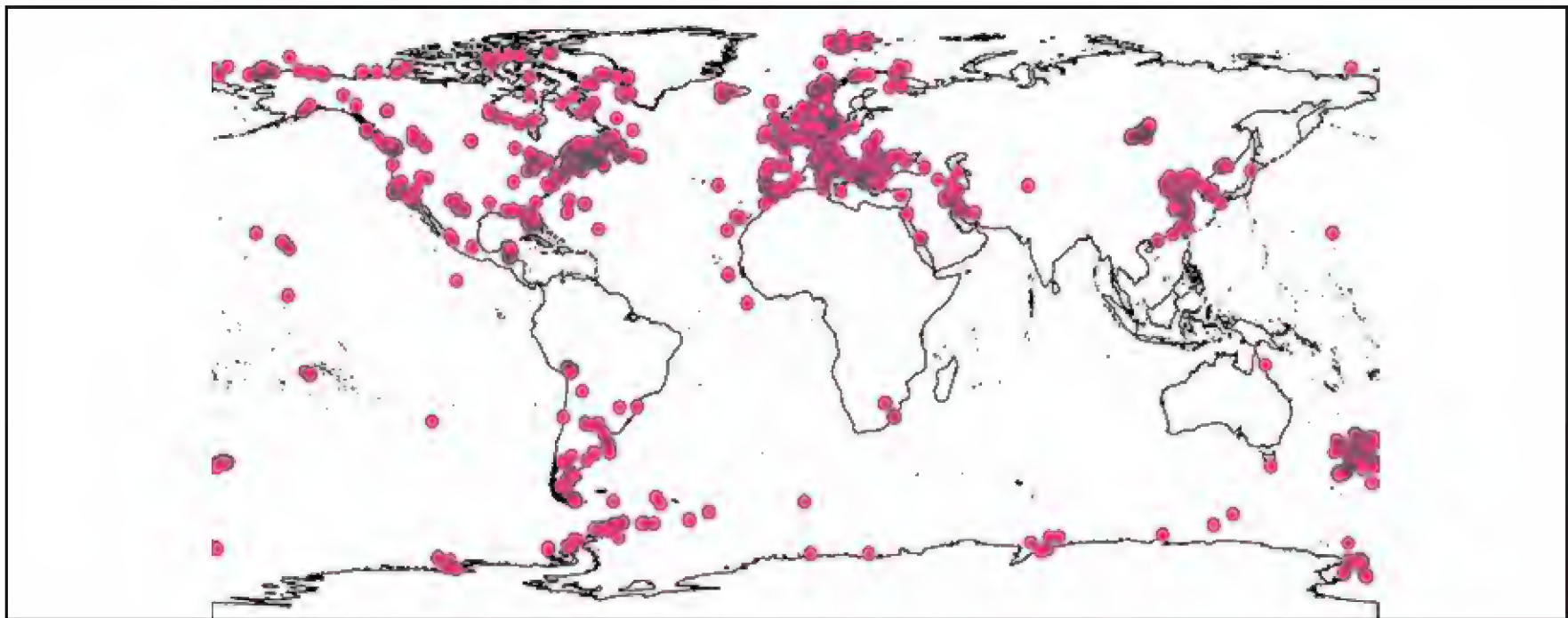


Figure 1. Collecting sites for amphipod records with GPS information in BOLD (Map built in SimpleMappr).

the global information pertaining to DNA barcoding, not only data generated by BOLD users. We gathered all public COI sequences available for Amphipoda into one dataset in BOLD. Various analytical tools readily available in BOLD were employed to summarize data and provide general statistics. Moreover, a gap analysis was performed on the barcode library by integrating available global species lists with BOLD to obtain the list of species yet to be barcoded. In the process, we identified taxonomic synonymies present in BOLD/GenBank.

RESULTS

Out of >6 million DNA sequences accumulated to date, Amphipoda covers only a small fraction (~27,000 sequences). Only 17,054 sequences were public in April 2017, half of them being mined from Genbank (9,014 sequences). Most records had some metadata available, at least the country of collection, except for the records mined from GenBank (which has less stringent metadata requirements for sequence submission). The available amphipod records have been collected in 78 countries, with Canada being the main source (3,090 records), while only 8,702 records had GPS details (Fig. 1). Approximately 200/17,054 sequences have been flagged to date as contamination, misidentification or as having stop codons (indicative of pseudogene amplification). The identified amphipods belonged

to 1,341 species, 373 genera and 108 families. The unidentified amphipods raised to 3,391/17,054. BOLD provided a total of 2,484 BINs for 17,054 sequences suggesting the existence of cryptic species, misidentification and/or lack of species names. The BIN discordance report available in BOLD showed that 249 BINs were discordant (i.e., one BIN included multiple taxa) across the entire database. This result is suggestive of misidentification, synonyms, potential errors (misspelled names), lack of the barcode gap (e.g., closely related species), introgression or hybridization.

CONCLUSIONS

New technological advances in sequencing methodology (e.g., next-generation sequencing, S.M.R.T. sequencing) will only increase the amount of molecular data produced, a subset of which will lack taxonomic identification. The resulting datasets, especially if employing BINs for clustering, may have important utility for various studies (e.g., molecular biodiversity assessments and monitoring). However, in order to have a reliable barcode database for species identification, there is a need for concerted efforts between classical taxonomists and barcoders. Such database with fully identified records could be used for inferring patterns in biogeography and biodiversity, and help make better predictions in the context of global change.

Catalogue of the Orders Amphipoda and Ingolfiellida (Crustacea Peracarida) from Brazil

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ABSTRACT

This catalog includes 360 amphipod species within 179 genera, 70 families, three suborders and two orders. The order Ingolfiellida has one record. The order Amphipoda includes the suborder Hyperiidea with 55 pelagic species, usually associated to gelatinous plankton. Suborders Amphilochidea and Senticaudata are represented by 270 benthonic, marine and/or estuarine species occurring from a diverse type of biogenic and non biogenic substrates. There are 35 non-marine amphipods reported from Brazil.

KEY WORDS

Catalog; Amphipoda; Brazil.

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INTRODUCTION

Amphipods *s.l.* are one of the most diverse and abundant groups among the Superorder Peracarida and currently there are nearly 10.000 described species (Horton et al., 2016). Recent revision proposed by Lowry & Myers (2017) treated the Ingolfiellida as an order and the order Amphipoda *s.s.* includes six suborders: Amphilochidea Boeck, 1871; Colomastigidea Stebbing, 1899a; Hyperiidea H. Milne Edwards, 1830; Hyperiopsidea Birstein & Vinogradov, 1955; Pseudingolfiellidea Lowry & Myers, 2012; Senticaudata Lowry & Myers, 2013. The group is mostly marine, with a worldwide distribution, wide range of bathymetric distribution (supralittoral to hadal zones) and generally is one of the main components of the marine macrofauna. Some families colonized the continental waters, including subterranean waters, caves and the terrestrial environment. The present catalog is an update from

Wakabara & Serejo (1998) including also information on the suborder Hyperiidea.

MATERIAL AND METHODS

This catalog documents the order Ingolfiellidea and order Amphipoda *s.s.* (suborders Amphilochidea, Hyperiidea and Senticaudata) that occur in Brazil with information on type material, type locality, geographic and bathymetric distribution, ecological notes and remarks when pertinent. Moreover, several amphipod records for Brazil were noticed to be misidentifications or dubious identification and will be treated separately.

RESULTS

This catalog includes 360 species within 179

genera, 70 families, three suborders and two orders. The suborder Hyperiidea includes 55 pelagic species, usually associated to gelatinous plankton. Suborders Amphilochoidea and Senticaudata are represented by 270 benthonic, marine and/or estuarine species occurring from a diverse type of biogenic and non biogenic substrates. Considering the bathymetric distribution, marine amphipods occur from the supralittoral to hadal depths. Excluding the hyperiids, there are 216 species (~ 80%) recorded for the Brazilian continental shelf (0–200 m) and 60 (~20%) for the deep sea zone (> 200 m). Only six species presented a eurybathic distribution, colonizing both shallow and deep sea zones (*Cephalophoxoides homilis* (J.L. Barnard, 1960), *Cuneimelita danielle* Senna & Serejo, 2012, *Harpiniopsis galera* J. L. Barnard, 1960, *Oediceroides cinderella* Stebbing, 1888, *Paracaprella pusilla* Mayer, 1890, *Phtisica marina* Slabber, 1769). There are 35 non-marine amphipods reported from Brazil. *Hyaella* S.I. Smith, 1874 is the restricted and dominant epigean group in South America, with 70 species described and with 22 species registered in Brazil, being 16 epigean and six hypogean (Gonzalez et al., 2006; Bueno et al., 2013; Cardoso et al., 2014). The family Artesiidae includes seven cave species of *Spelaeogammarus* Brum, 1975 from Bahia and Minas Gerais (Koenemann & Holsinger, 2000; Bastos-Pereira & Ferreira, 2017) and the Bogidiellidae are represented by one cave-dwelling and one subterranean species (Koenemann & Holsinger, 1999). More recently, Fišer et al. (2013) described two new cave species for Rio Grande do Norte, *Seborgia potiguar* Fišer, Zigmajster et Ferreira, 2013 (Seborgiidae) and *Potiberaba porakuara* Fišer, Zigmajster et Ferreira, 2013 (Mesogammaridae). The family Talitridae is unique among the amphipods in colonizing the terrestrial environment occurring in caves and among the forest litter. Among the landhoppers (truly terrestrial), only two introduced species, *Talitroides alluaudi* (Chevreux, 1896) and *T. topitotum* (Burt, 1934) have been found in the Atlantic forest bioma, urban parks and sylviculture plantations from the southeast and south of Brazil (Nascimento & Serejo, 2016).

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On the functional role of large and small scavenging amphipod species (Lysianassidae) in Potter Cove food web (Antarctica)

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ABSTRACT

Investigations on complementary influences of scavenging amphipods in carrion decomposition are presented and their contributions to the local food web are discussed.

KEY WORDS

Succession; carrion decomposition; Southern Ocean; biomass transformation.

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Scavenger guilds are present in all ecosystems and are composed of a variety of co-existing species in the same habitat. Both biotic and abiotic parameters play a major role in niche differentiation between competing scavenger species for the same resources. Niche segregation between carrion-feeders can manifest in morphological and behavioural adaptations. Well-known terrestrial examples of co-existing scavengers are the Old World vultures. They can be classified into functioning types where large species are tearers, medium-sized pullers and small species are considered as pickers during carrion feeding (Campbell, 2015; Kruuk, 1967).

In marine environments, the first successional stage of carcass decomposition is often dominated by quick and highly motile scavenger species which predominantly are amphipod species from the superfamily Lysianassoidea (e.g. Anderson & Bell, 2016; Premke et al., 2006; Smith & Baco, 2003). To date the intra-guild interactions of Antarctic scavenging amphipod species (Lysianassidae) during

carcass decomposition remain broadly unstudied.

In the present study we investigated the interaction between co-existing scavenging lysianassid species sampled in Potter Cove (King George Island/Isla 25 de Mayo, Antarctica) by means of laboratory feeding trials. The objective was to identify the functional role of large and small lysianassid species while feeding on carrion. In this way we aimed to achieve information whether the speed of degradation of fish carcasses depends solely on the biomass or numbers of amphipods present or if an optimal mixture of large and small amphipods would further increase biomass turnover.

These synergistic effects, if any, were studied among the relatively large-sized lysianassid species *Waldeckia obesa* (Chevreux, 1905) and the smaller lysianassid species *Cheirimedon femoratus* (Pfeffer, 1888), *Orchomenella rotundifrons* (Barnard, 1932) and *Hippomedon kergueleni* (Miers, 1875) when feeding on entire carcasses of local fish (Nototheniidae: *Notothenia* spp.). Behavioural aspects were

analysed visually and recorded, and the decomposition of fish carcass was measured in terms of consumption rates of amphipods in absence or presence of large and small amphipod species.

As *C. femoratus* is the only scavenging amphipod species distributed in high abundances both in outer and inner Potter Cove (Seefeldt et al., 2017), feeding rates on different food items (macroalga: *Palmaria decipiens* (Reinsch, 1888) R.W. Ricker 1987; fish: *Notothenia* spp.) were estimated to assess the species contribution to biomass transformation in the local food web. We discuss morphological and behavioural adaptations and compare our findings with other scavenging guilds and their importance for ecosystem functioning in general.

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Cataloguing carrion-feeding amphipods (Lysianassoidea) in the Filchner area: A unique area in the Weddell Sea (Antarctica)

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ABSTRACT

We investigated the species diversity of carrion-feeding amphipods (Lysianassoidea) of the Filchner area (Southern Ocean) using molecular and morphological method.

KEY WORDS

Southern Ocean; scavengers; biodiversity ‘hotspot’; DNA barcoding; taxonomy.

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The assessment of scavenging lysianassoid amphipod species diversity in the Southern Ocean is largely incomplete and patchy because of error-prone morphological species identification for lysianassoids and the inaccessibility of unnavigable regions with difficult sea ice conditions. Accurate biodiversity inventories, however, are of increasing importance nowadays in order to monitor, protect and conserve unique ecosystems in the light of ongoing and future environmental changes. The Filchner area in the south eastern Weddell Sea is i.a. characterized by its all year round heavy sea ice conditions, occurrence of supercooled waters and is considered to be a biological ‘hotspot’ compared to other regions of the Weddell Sea (Knust & Schröder, 2014). The availability of high biomass of nekton and marine mammals in the Filchner area may imply the presence of a remarkable amphipod scavenger fauna, which so far has remained unstudied.

The aim of this study was to fill the knowledge

gap of species composition of carrion-feeding amphipods throughout the Filchner area by applying an integrated approach of in-depth morphological identifications and DNA barcoding. Lysianassoid amphipods were collected and investigated (COI) from 16 different stations of the eastern and southernmost parts of the Weddell Sea deploying 5 different sampling devices, including the southernmost baited traps yet deployed in the Weddell Sea. We discuss advantages and drawbacks of the use of baited trap in marine biodiversity research and the importance of the amphipod scavenger guild in this unique ice-bound ecosystem of the Weddell Sea.

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Intersexual differences of thermal sensitivity and heat shock response proteins in Baikal endemic amphipods

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ABSTRACT

In two congener Baikal amphipods *Eulimnogammarus verrucosus* (Gerstfeldt, 1858) and *E. cyaneus* (Dybowski, 1874) (Eulimnogammaridae), the sexually dimorphic thermal sensitivities and cellular heat shock responses were estimated. Higher sensitivity of the females from both species to heat shock than the males, was found. The results indicate that *E. cyaneus* females are better equipped for an acute thermal stress response, than *E. verrucosus* females, which can be explained by the season of reproduction of the studied species.

KEY WORDS

Hsp70; proteome; Amphipoda; stress response; thermotolerance; Baikal.

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Temperature, as an abiotic factor, has the potential to alter physiology of organisms (Hochachka & Somero, 2002). The ability of an immediate adaptation or stress-response is essential for aquatic ectotherms that are unable to self-regulate their core body temperature. It is especially important for cryophilic stenotherms, such as those endemic to Lake Baikal (Siberia), because their proteomes have evolved to function most efficiently at lower temperatures (e.g. <10 °C). To provide comprehensive information about the potential physiological responses of species given Lake Baikal's rapidly warming surface temperatures, studies of intraspecific differences in thermotolerance are essential. In the present study, we investigated intersexual variation of thermal sensitivity (i.e., Hsp70 levels) and proteomic heat shock response in two congener Baikal littoral amphipods *Eulimnogammarus verrucosus* (Gerstfeldt, 1858) and *E. cyaneus* (Dybowski, 1874)

(Eulimnogammaridae), the most abundant species in the littoral zone of Lake Baikal (Timoshkin et al., 2009).

To determine intraspecific sensitivity to an acute thermal stress and the heat shock response, amphipods were incubated at the previously determined temperature of 50% mortality of the mixed adult population for each species. Proteins of stress-response were estimated using the combined 1D and 2D SDS-PAGE, western blotting and mass-spectrometry (MALDI-TOF/TOF).

The results indicated the higher sensitivity of the females from both species to heat shock than the males. For *E. cyaneus*, the LT50time was calculated as 13.6 h for females and 35 h for males. For *E. verrucosus*, LT50 was estimated as 7.9 h for females, while the males lived significantly longer (e.g., LT50 = 26.5 h). Higher sensitivity of female to heat shock can be explained by the increased metabolic energy demand for oogenesis compared to the less

energy demanding spermatogenesis (Buikema & Benfield, 1979).

The basal levels of Hsp70 in the controls for each of the two species were significantly different. The Hsp70 level was 2.6 times higher in the male *E. cyaneus* than in the male *E. verrucosus* ($P < 0.001$). Female *E. cyaneus* contained 11.6 times higher Hsp70 levels than the female *E. verrucosus* ($P < 0.001$). The heat shock (1 h) and recovery (3 h) significantly induced Hsp70 accumulation in the *E. verrucosus* males ($P = 0.01$). In the thermotolerant *E. cyaneus*, a slightly higher basal level of Hsp70 was found in the female controls compared to the males. No significant increase in Hsp70 was found in this species when it was exposed to heat shock (1 h) and the subsequent recovery (3 h). Shown strict difference in the intersexual level of Hsp70 indicates that female *E. verrucosus* are more vulnerable to temperature increases since they breed during the winter and lack the necessary regulation of Hsp70 activation during the temperature increase. Conversely, females of the thermotolerant summer-reproducing species *E. cyaneus* activate the regulation of Hsp70 induction during their breeding to sustain summer temperatures in the upper littoral zone.

Up to 15 differently expressed protein spots were found in the gels from males and females of both studied species. Heat shock and the subsequent recovery altered the optical density (OD) of 13 protein spots in *E. cyaneus* females. Only the OD for one protein spot decreased on the gels containing the *E. cyaneus* males. Seven protein spots had a significantly decreased OD among the female *E. verrucosus*, whereas in the males only one protein spot showed a slightly increased OD. Using public databases (NCBI, SwissProt), we determined the

identity of 18 protein spots in the gels. Among the identified proteins, hemocyanin and arginine kinase had an increased OD in the *E. cyaneus* females, and cytosolic malate dehydrogenase was increased in the *E. verrucosus* males that were exposed to heat shock and recovery. Bringing together the Hsp70 and proteomic results, we can consider that *E. cyaneus* females are better equipped for an acute thermal stress response, than *E. verrucosus* females, as indicated by the presence of higher energy metabolic proteins following a heat shock response together with more efficient molecular mechanisms of an Hsp70-mediated heat shock response.

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First assessment of the epifauna associated with macroalgae of the vermetid reef along the coasts of Favignana Island (South Tyrrhenian Sea)

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ABSTRACT

With this study we provide a first baseline assessment of the epifauna associated with the macroalgae of the vermetid reefs present along the coasts of the Island of Favignana (Egadi Islands Marine Protected Area, Italy). A total of 14 taxa were identified. Epifaunal assemblages differed according to structure and composition of algal communities. The amphipod group presented the highest number of individuals. The tubicolous species *Ampithoe ramondi* Audouin, 1826 (Ampithoidae) was the most abundant species.

KEY WORDS

Fauna biodiversity; Favignana Island; macroalgae; southern Tyrrhenian Sea; vermetid reef.

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INTRODUCTION

Vermetid reefs are bioconstructions built up by the gastropod mollusc *Dendropoma cristatum* (Biondi, 1859) (Vermetidae) in association with some coralline algae such as *Neogoniolithon brasica-florida* (Harvey) Setchell et Mason. In Sicily, large and more or less continuous vermetid reefs are present along the entire coast facing north/northwest between Zafferano Cape and the coast of Trapani with the Marine Protected Area (MPA) “Egadi Islands” (Chemello, 2009). These bioconstructions play a fundamental structural role, as they protect coasts from erosion, regulate sediment transport and accumulation, serve as carbon sinks, make the habitat more complex and heterogeneous and provide numerous habitats for animal and vegetal species thus increasing intertidal biodiversity (Chemello, 2009). Macroalgae may have an important role in structuring associated epifaunal assem-

blages (Jones & Thornber, 2010). The aim of this study was to analyse the epifauna associated with the macroalgae inhabiting the out margin of the vermetid reefs along the coasts of Favignana Island (Egadi Islands Marine Protected Area). A recent analysis of the vermetid reefs along the coasts of Favignana Island displayed at least three local patterns (Balistreri et al., 2015). Since the outer margin of the diverse typologies was inhabited by different macroalgal communities, we hypothesized that epifaunal assemblages differed in relation to the structure and composition of macroalgal community. We report here the first results of this study.

MATERIAL AND METHODS

The study was carried out in summer 2016 in ten areas chosen along the coasts of Favignana Is-

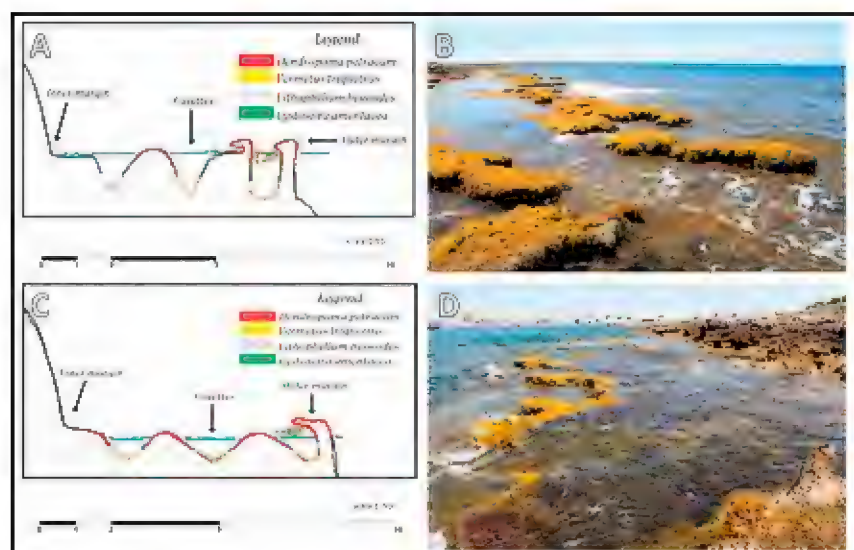


Figure 1. The analysed vermetid reefs. A. Scheme of the pattern 1. B. Faraglione. C. Scheme of the pattern 3. D. Arre Turino

land (Egadi Islands MPA), located approximately five kilometers from the western coast of Sicily (South Tyrrhenian Sea, Italy). In this paper we considered 4 representative areas, 2 characterized by a vermetid reef belonging to the pattern 1 (Faraglione and Pozzo) and 2 by a vermetid reef belonging to the pattern 3 (Arre Turino and Cala Rotonda) (Fig. 1) (see Balistreri et al., 2015), where the outer margin was characterized respectively by mediolittoral (*Laurencia*) and infralittoral (*Cystoseira*, *Dictyopteris*) algal species. For each site, three random replicates were taken by scraping off the substratum within a 20×20 cm frame from the outer margin of the reef. The samples were washed through 0.5 mm sieves and the algae were then searched thoroughly for any remaining animals under a stereomicroscope. After a sorting process the isolated animals were counted. All animals and algae were identified to the lowest taxonomic level feasible.

RESULTS AND CONCLUSIONS

A total of 14 taxa were identified, belonging to crustacean decapods (1), echinoderms (1), molluscs (8), polychaetes (2) and amphipods (2), the last one presenting the highest number of individuals. At Faraglione and Arre Turino the highest number of amphipods was found (Table 1).

Biodiversity was higher at Cala Rotonda and Arre Turino, whereas abundances were higher at Faraglione and Arre Turino (Table 1). Differences in abundances seem to be linked to the coverage, shape and structural complexity of algae (Cacabelos et al., 2010). Indeed, Faraglione and Arre Turino were characterized by 100% algal coverage and by the dominance of algae with a high structural complexity (*Laurencia* and *Cystoseira*, respectively). At Cala Rotonda, the dominance of a sheet-like alga (*Dictyopteris*) would explain the lower number of individuals. At Pozzo, characterized by 30% algal coverage (*Laurencia* was the dominant species), the lowest diversity was found.

The amphipod diversity was very low in all sites, only two herbivorous species were found: *Elasmopus pocillimanus* (Spence Bate, 1862), a free-living species, and *Ampithoe ramondi* Audouin, 1826, a tubicolous species. *Ampithoe ramondi* was the only species found at Arre Turino and the most abundant species at Faraglione. This species prefers areas with high hydrodynamism such as the vermetid reef and preferentially consumes algae with a higher structural complexity (Jacobucci & Leite, 2014).

The present findings suggest that the total abundance and species richness of epifauna are mainly related to the structure and composition of algal

Area	Algal coverage	Dominant alga	amphipods	decapods	echinoderms	molluscs	polychaetes
Arre Turino	100%	<i>Cystoseira</i> (100%)	27	2		4	10
Cala Rotonda	100%	<i>Dictyopteris</i> (90%)	13		9	4	2
Faraglione	100%	<i>Laurencia</i> (90%)	30	1		10	
Pozzo	30%	<i>Laurencia</i> (25%)	2			8	

Table 1. Data of macroalgae and the total number of individuals of epifaunal taxa recorded in the studied vermetid reefs.

communities and to the structural complexity of algae. It is presumable that structurally complex algae may create more physical refuges for epifauna than algae with a lower level of complexity.

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Two new records of carnivorous amphipods (Amphipoda Gammaridea Lysianassoidea) collected from Korean waters

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ABSTRACT

Two carnivorous lysianassoid amphipods collected from Korean waters. These species belonging to the genera *Anonyx* Krøyer, 1838 and *Orchomenella* Sars, 1890. Their morphological characteristics are explained in this study.

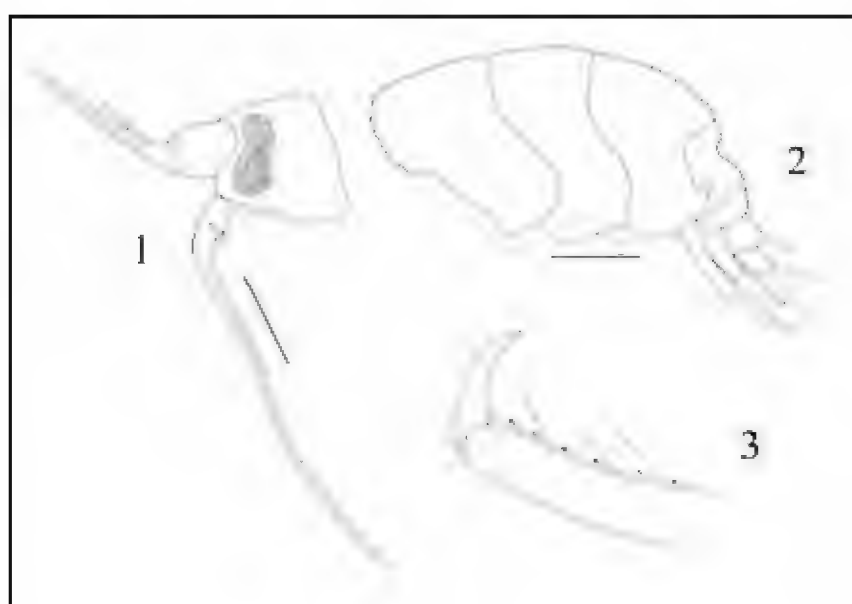
KEY WORDS

Amphipoda; Lysianassoidea; new record species; Korea.

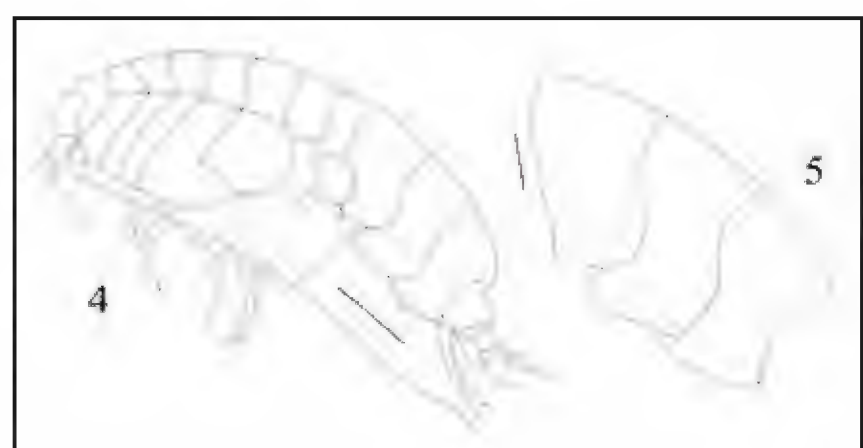
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Two lysianassoid amphipods, *Anonyx* sp. (Fig. 1–3) and *Orchomenella* sp. (Fig. 4–5) were collected by a bait trap and fishing net from Korean waters. The genus *Anonyx* Krøyer, 1838 includes 49 species worldwide but has not been reported from Korea. The collected *Anonyx* species has the following characters; 1) gnathopod 1, propodus slightly



Figures 1–3. *Anonyx* sp., male, 13.8 mm. 1: head, lateral, 2: epimeral plates 1-3 & urosome, 3: pereopod 3, propodus. Scale bars: 1.0 mm (1, 2).



Figures 4–5. *Orchomenella* sp., male, 7.3 mm. 4: habitus, lateral, 5: epimeral plates 1-3. Scale bars: 1.0 mm (4), 0.4 mm (5).

ghtly narrowed distally, dactylus without accessory tooth; 2) pereopods 3–4, posterior edge of propodus with a row of long setae each accompanied by a spine; and 3) uropod 2, inner ramus unconstricted. Our Korean specimens are morphologically similar to *A. schefferi* Steele, 1986 and *A. laticoxae* Gurjanova, 1962. The genus *Orchomenella* Sars, 1890 consists of 31 species worldwide but only two species, *Orchomenella japonica* Gurjanova, 1962 and *Orchomenella obtusa* (Sars, 1891) have been re-

ported in Korea. The collected *Orchomenella* species has the following characters; 1) pleonal epimeron 3 characteristic in form, serrated posterodistally; 2) pereopods 3–5, basis shorter than the rest of the appendages, the hind margin minutely serrated; and 3) uropod 3, inner ramus subequal to the proximal article of outer ramus. Our Korean specimens are morphologically similar to *Orchomenella littoralis* Nagata, 1965 and *Orchomene breviceps* Hirayama, 1986.

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10-year monitoring of alien amphipods in Belarus: state of the art

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ABSTRACT

The revision of publications that appeared during 10 years of monitoring distribution of alien amphipods in Belarus was done. Some details of records and distribution of alien species were corrected, e.g. *Obesogammarus crassus* (Sars G.O., 1894) and *O. obesus* (Sars G.O., 1894) were found for the first time in the Dnieper River in 2011 instead of 2007 and 2008 respectively as was mentioned in the first checklist of alien species of Belarus. Presence of *E. trichiatus* was confirmed by DNA barcoding. New records of *Echinogammarus trichiatus* (Martynov, 1932) and *Chelicorophium curvispinum* (G.O. Sars, 1895), *C. robustum* (G.O. Sars, 1895) were pointed out in Belarus. Three species, *Dikerogammarus villosus* (Sowinsky, 1894), *Pontogammarus robustoides* (Sars, 1894) and *C. curvispinum*, were included in the Black book of invasive alien animals of Belarus published in 2016.

KEY WORDS

Alien amphipods; monitoring; Belarus.

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Alien species are among the main biological threats to biodiversity. Studies upon alien fauna composition and abundance, introduction pathways and vectors, impact on native communities play a significant role in the development of a national invasive alien species management plan and/or a national invasive alien species strategy. Our study of alien species composition, abundance and their distribution along the main river of Belarus has started in 2006. The first article directly addressing alien amphipods of Belarus was published by Mastitsky & Makarevich (2007). Since then, monitoring of alien species has been conducted as a part of the National Biodiversity Strategy and Action Plans for 2016–2020 to facilitate the achievement of Aichi Biodiversity Target 9. The aim of the present study was to analyze the published and own data

for 10 years of monitoring of alien amphipods and to correct some data in the recently published articles.

Nowadays, nine species of Ponto-Caspian amphipods are known to have established and widely spread in the water bodies of Belarus (Mastitsky & Makarevich, 2007; Karatayev et al., 2008; Semenchenko et al., 2009; Makarenko & Vezhnovets, 2014; Lipinskaya & Makarenko, 2015; Semenchenko et al., 2016). The main hotspot of alien amphipods in Belarus is in the Dnieper River near Niznie Zhary vill., where all the nine alien amphipods were recorded.

Since the first record of *Dikerogammarus villosus* (Sowinsky, 1894) and *Dikerogammarus haemobaphes* (Eichwald, 1841) in the Dnieper River in 2006 (Mastitsky & Makarevich, 2007), these spe-

cies spread across the main waterways. *D. villosus* spread in the Dnieper River up to Rechitsa town, in the Sozh River up to Chechersk town, in the Pripyat River up to the Pina River mouth, in the Dnieper-Bug Canal and in the Muchavets up to Brest town (Makarenko & Vezhnovets, 2014; this study).

Dikerogammarus haemobaphes is the most widespread alien species in Belarus. It spread across the Dnieper River up to Bujnichi vill. (Makarenko, 2014 unpublished; this study), in the Berezina River up to Bobrujsk town, in the Sozh River up to Chechersk town, in the Pripyat River up to Pinsk town, across the Pina River up to Duboy vill., across the Horyn River up to Belousha vill. and across the Muchavets up to Brest town (Makarenko & Vezhnovets, 2014; this study). The presence of *D. bispinosus* was not proved by DNA barcoding (Lipinskaya 2017, unpublished).

For the first time, *Chelicorophium curvispinum* (G.O. Sars, 1895) was found in the Neman River near Lunno vill. in 2014 (Semenchenko, 2014 unpublished), while it was recorded in the Pripyat River in 1914 (Wolski, 1930) and in the Dnieper River in 1965 (Vladimirova et al., 1965). Its uppermost site in the Dnieper River is near Bykhov town (Lipinskaya, 2010 unpublished), and in the Pripyat River it is near Pinsk (Makarenko, 2015 unpublished), in the Dnieper-Bug Canal (2015), in the Berezina River - near Bobrujsk town (Lipinskaya, 2016 unpublished), in the Sozh River - Vetka town (Makarenko & Vezhnovets, 2014), in the Neman River - near Lunno vill. (Semenchenko, 2015 unpublished), in the Muchavets River is near Brest town (Makarenko, 2015).

Chelicorophium robustum (G.O. Sars, 1895) was considered inhabiting only the Dnieper River near Nizhnie Zhary vill. (Semenchenko et al., 2009) until this species was found by Makarenko in 2014 and by Lipinskaya in 2016 in the Pripyat River near Narovlja town.

Pontogammarus robustoides (Sars, 1894) was revealed in the Dnieper River near Kholmech vill. in 2006 (Mastitsky & Makarevich, 2007) and in the Pripyat River - near Narovlya in 2014 (Makarenko, 2014 unpublished) and in the Sozh - near Vetka town in 2013 (Makarenko & Vezhnovets, 2014).

Obesogammarus obesus (Sars G.O., 1894) and *Obesogammarus crassus* (Sars G.O., 1894) were found for the first time in the Pripyat River in 2008 (Semenchenko & Vezhnovets, 2008) and in the

Dnieper River in 2011 (Semenchenko et al., 2013). Since then, *O. obesus* spread along the Pripyat River up to Mozir town (Lipinskaya, 2016 unpublished), along the Dnieper River up to Rechitsa town (Lipinskaya, 2016 unpublished) and along the Sozh River up to Vetka town (Makarenko & Vezhnovets, 2014). *O. crassus* spread along the Dnieper river up to Novij Byhov vill. (Lipinskaya, 2010 unpublished; this study), along the Pripyat River up to Korobje vill. (Makarenko & Vezhnovets, 2014). Also, *O. crassus* was found in the Horyn River near Rechitsa vill. (Makarenko, 2013 unpublished), in the Pina River and in the Sozh River near Vetka town (Makarenko & Vezhnovets, 2014).

Echinogammarus trichiatus (Martynov, 1932) was revealed only in the Dnieper River near Nizhnie Zhary vill. in 2010 (Lipinskaya, unpublished). This identification was proven by DNA barcoding (Lipinskaya & Makarenko, 2015). The species was found again at the same site in 2011 (Makarenko & Vezhnovets, 2014) and in 2016 (Lipinskaya, unpublished).

For the first time, *Echinogammarus ischnus* (Stebbing, 1899) was found in the Dnieper River in 2006 (Mastitsky & Makarevich, 2007) and in the Pripyat River in 2007 (Semenchenko et al., 2009). The upper site of *E. ischnus* distribution in the Dnieper River is near Rechitsa town, in the Pripyat River it is near Pinsk town, in the Sozh River is near Vetka town and in the Horyn River is near Belousha vill. (Makarenko & Vezhnovets, 2014; this study).

Three out of nine alien species, *D. villosus*, *P. robustoides* and *C. curvispinum*, were included in the Black Book of alien invasive species of Belarus (Semenchenko, 2016).

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Macrofauna associated with the bryozoan *Biflustra grandicella* (Canu et Bassler, 1929) on the Brazilian coast

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ABSTRACT

The fauna associated with the bryozoan *Biflustra grandicella* (Canu et Bassler, 1929) (Membraniporidae) in southeast Brazilian coastal waters was analyzed. Results show that the colony constitutes a suitable environment for tube-dwellers detritivorous species. Besides the dominance of amphipod *Podocerus brasiliensis*, it is remarkable the identification of the exotic species *Stenothoe gallensis* for the second time.

KEY WORDS

Amphipoda; Bryozoa; associated fauna.

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INTRODUCTION

Marine bryozoans with colonies of arboreal type provide a substrate for colonization by various organisms, offering shelter, protection and food (Morgado & Tanaka, 2001). The objective of this study was to analyse the macrofauna associated with the bryozoan *Biflustra grandicella* (Canu & Bassler, 1929) (Membraniporidae) recognized as an invasive species by Tilbrook (2012).

MATERIALS AND METHODS

The colony of *B. grandicella* was collected in Araçá Bay, São Sebastião, on the North coast of the State of São Paulo, Brazil. The bay is located in the central area of the São Sebastião Channel, near the Port of São Sebastião. It is part of a marine protected area and has one of the richest diversities of the Brazilian coast (Amaral et al., 2016). The colony of *B. grandicella* was found in March 2014 at the depth of 8 m, overgrowing a rope (Fig. 1), and was

of interest due to its arborescent growth. The colony was fixed in 70% alcohol and the associated macrofauna was removed and identified to species level.

RESULTS AND CONCLUSIONS

The macrofauna associated with *B. grandicella* was represented by 10 species, six amphipods and four polychaetes, totalling to 7,672 individuals. The amphipod *Podocerus brasiliensis* (Dana, 1853) (Podoceridae) (Fig. 2) was the dominant species with 7,094 individuals identified, comprising 92.5% of the entire community. The other amphipods *Ampithoe cf. ramondi* Audouin, 1826, *Stenothoe gallensis* Walker, 1904 (Stenothoidae), *Erichthonius brasiliensis* (Dana, 1853) (Ischyroceridae), *Elasmodon pecteniscrus* (Bate, 1862) (Maeridae) and *Jassa slatteryi* Conlan, 1990 (Ischyroceridae), with 420, 100, 31, 6 and 2 individuals, respectively, corresponded to 7.3% of the macrofauna, while only 1.2% consisted of polychaetes, represented by *Leodice rubra* (Grube, 1856) (Eunicidae), *Pseudonereis*



Figure 1. Colony of the bryozoan *Biflustra grandicella*.



Figure 2. *Podocerus brasiliensis*, dominant amphipod of macrofauna associated with *Biflustra grandicella*.

palpata (Treadwell, 1923) (Nereididae), *Branchiomma* sp. (Sabellidae) and *Lepidonotus* sp. (Polynoidae), with 12, 4, 2 and 1 individuals respectively.

Our results are similar to those obtained by Conradi et al. (2000), where amphipods were the most abundant group of the fauna found in association with *Bugula neritina*, but different from the results found by Morgado & Tanaka (2001) where polychaetes were more numerous in macrofauna inhabiting colonies of *Schizoporella unicorni*. The difference in abundance and composition of the associated species communities may be a result of host morphology, temporal variation and biological interactions between species. The occurrence of *S.*

gallensis in the colony suggests that this exotic species remains present on the north coast of São Paulo, 16 years after its first record. It should be noted that adult individuals, young and ovigerous females were found. The record of *S. gallensis* in 2001 from the coast of São Paulo (Leite et al., 2011) and this new record in Araçá Bay may indicate that its introduction occurred through association with biofouling of ships. The arborescent colony of *B. granciella* species, with suspension habits, constitutes a favourable environment for tubicolous detritivorous species. The analysis of a greater number of samples would be ideal.

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Amphipods assemblages associated with non-indigenous macrophytes in Monastir coast (Eastern Tunisia)

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ABSTRACT

A comparison between amphipods assemblages inhabiting non-indigenous and native macrophytes was conducted along the Monastir Bay in the eastern coast of Tunisia. Samples of Amphipoda from the non-indigenous seagrass *Halophila stipulacea*, the algae *Caulerpa racemosa* and *Asparagopsis taxiformis* and the native seagrass *Cymodocea nodosa* and the algae *Sargassum vulgare* were collected using a quadrat of 0.2x0.2 m². An overall reduction in the number of species and abundance of peracarids was found to be associated with non-indigenous macrophytes

KEY WORDS

Peracarids; *Halophila stipulacea*; *Caulerpa racemosa*; *Asparagopsis taxiformis*.

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The central Mediterranean Sea is one of the regions worldwide most severely affected by alien marine invasions (Zenetos et al., 2012). With over 1,400 km of coastline, and located at the crossroads between the different sub-basins, the Tunisia occupies a key position. The Tuniso-Sicilian Strait is the passageway from east to west, and is crucial in the analysis of the spread of alien species introduced into the Mediterranean Sea.

The composition of amphipods assemblages is often linked to macrophytes coverage, where species find refuge from predators and a availability of food resources. Changes on the composition of macrophytes could affect associated fauna assemblages. Sghaier et al. (2016) reported a total of 27 marine alien macrophytes along the Tunisian coast and many of them were reported in Monastir coast.

The aim of this paper was to compare the amphipods assemblages inhabiting non-indigenous and native macrophytes in the Monastir Bay.

Monastir (N35°47' E10°49') is located on the eastern coast of Tunisia in the Mediterranean Sea. Sampling was conducted in May 2017 a quadrat of 0.2 m perside. Three replicates were sampled for each studied macrophyte. Algae or seagrass samples were taken at depth of 0.5 to 2 m. At each sample site, macrophytes were scraped and collected directly in a bag (net size 0.3 mm). Macrophytes and the associated fauna were fixed in 70% alcohol, sorted and identified in the laboratory. Macrophytes were dried in 70°C for 48h, and weighed. The density of each species was compared to the surface and to the dry weight per 100g of macrophyte. The number of species (S), the total abundance (N), the mean density, the diversity index (H', log₂), and Pielou's evenness (J) were calculated for each replicate

A relatively high number of amphipod species have been recorded in macrophytes in the Monastir Bay. A total of 23 species were reported, whereas the number of species in each macrophyte species

ranged from 5 to 14 species. This result is comparable to other area in Tunisian coast at the same depth and in the same period of the year (Zakhama-Sraieb et al., 2011).

A group of species belonging to *Elasmopus*, *Maera* and *Stenothoe* genera were present over most of the studied habitats. The group of collected amphipods were common on photophilic algae of shallow waters within the Mediterranean Sea (Ruffo, 1982; 1993).

Number of species varied from a minimum of 5 species in the non-indigenous species *Halophila stipulacea* to a maximum of 14 species in the native species *Sargassum vulgare*. The composition of amphipod community varied in function of considered macrophyte. The highest values of amphipods density were recorded in *Sargassum vulgare*. The total abundance of amphipods increased within the native macrophytes. Diversity index and Evenness were lowest in the non-indigenous species compared with the native ones.

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An investigation of amphipods associated with sea urchins from Korea

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ABSTRACT

This study was conducted to investigate the amphipod species associated with sea urchins from Korea and relationships among these invertebrate species.

KEY WORDS

Amphipoda; association; sea urchins; Korea.

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Amphipod species associated with echinoderms have been found from all classes of echinoderms worldwide (Vader, 1978; Berge et al., 2004). The associations between amphipods and echinoderms are divided into five different categories based on the nature of the associations. The first category includes specialists, where amphipod species are obligate associates. The obligate species of amphipods feed on host tissues or secretion. In the second category, amphipod species play a role as scavengers. A number of amphipods are regularly found and they feed on dead or damaged sea urchins. In the third category, the amphipods, as energy commensals, not only get protection, but also obtain oxygen and food from the currents by burrowing sea urchins. The fourth and fifth categories include starfish associates and strays, respectively. The starfish associations are found especially among the caprellid amphipods (Vader, 1978; Berge et al., 2004).

The present study was conducted to investigate the amphipod species associated with sea urchins from Korea and to understand relationships between the amphipods and their host sea urchins.

Four species of sea urchins on the Korean coast, *Strongylocentrotus nudus*, *S. pallidus*, *Heliocidaris crassispina*, and *Diadema setosum*, were selected as the target hosts for this study. An unknown species of the genus *Dactylopleustes* Karaman et J.L. Barnard, 1979 was collected from the surface of two sea urchins, *Strongylocentrotus nudus* and *S.*

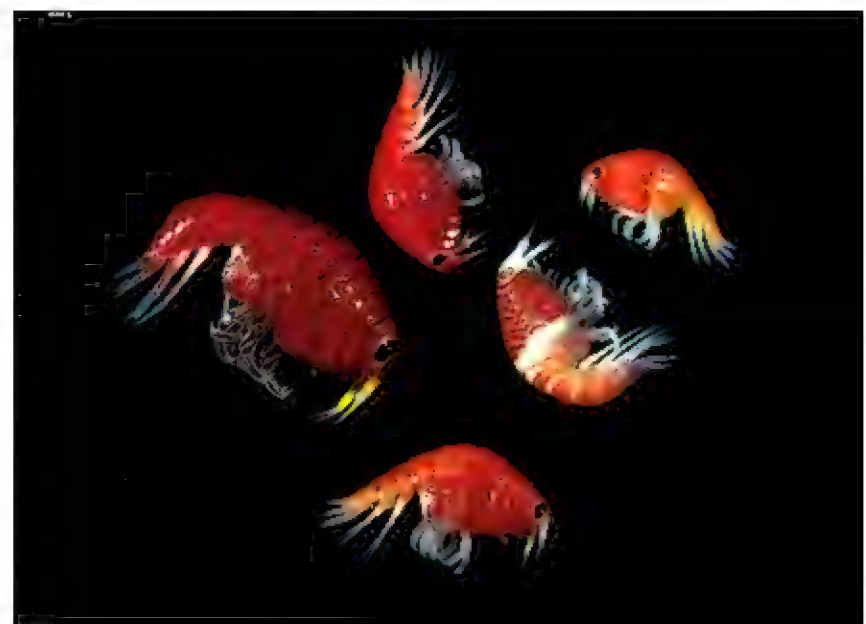


Figure 1. Photograph of the amphipod species of the genus *Dactylopleustes*.

pallidus (Fig. 1). The species of *Dactylopleustes* has subchelate dactyli on their pereopods. These specialized appendages are considered to be adapted to grasp urchin spines. In this study, we discuss the host specificity and distribution between species of *Dactylopleustes* and sea urchins.

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The effect of turbidity on the diversity of amphipods inhabiting *Sargassum* in Kuwait coastal waters with notes on their abundance, composition, and distribution: a preliminary study

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ABSTRACT

The distribution and abundance patterns of the amphipods associated to *Sargassum* species were studied along the Kuwait coastal waters. Physicochemical parameters were measured, focusing on turbidity.

KEY WORDS

Amphipods; turbidity; diversity; Kuwait waters.

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The distribution and abundance patterns of the amphipods associated to *Sargassum* species were studied on a spatial scale along the Kuwait coastal waters. Macroalgae, particularly *Sargassum* sp. are known to host high biodiversity of marine fauna, especially amphipods, when compared to non-vegetated habitats. Amphipods are an important component in the food web being consumed at different stages of fish life cycle. *Sargassum* was sampled from four different coastal stations within the subtidal zone of Kuwait to determine amphipod species composition and abundance (Fig.1). Physicochemical parameters like temperature, salinity, turbidity, pH and dissolved oxygen, were also measured at the time of sampling in each station, focusing on turbidity as a primary factor that can promote variation in the faunal distribution. The objective of this study was to test the effect of different physicochemical factors on amphipod biodiversity in *Sargassum* communities. The most abundant amphipods were represented by *Maxillipius rectitelson*

Ledoyer, 1973, *Erichthonius* cf. *forbesii* Hughes et Lowry, 2006 and *Podocerus* sp. On the other hand, from the measured physicochemical parameters,



Figure 1. Sampling Stations (Green Island, Salmiya, Failaka Island, and Khiryan) in Kuwait waters where *Sargassum* was collected.

only turbidity had a significant effect on the biodiversity of amphipods ($F_{1,6} = 19.35$, $p = 0.0046$) with a negative relationship based on general linear model test. The amphipod abundance was the highest with low turbidity in Salmiya, in comparison to high turbidity at Failaka and Green Island. Mean-

while, amphipods diversity varied among locations, being highest in Sargassum area of Salmiya and lowest in Failaka Island. In terms of species richness, Salmiya Station contained 27 species in its *Sargassum* area, while only few of them were found in the other stations.

A network of Italian amphipodologists in the frame of MOTax (Marine Organisms Taxonomy) at the Stazione Zoologica Anton Dohrn di Napoli (Italy)

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ABSTRACT

A proposal for a network of Italian amphipodologists among the activities of the Marine Organisms Taxonomy Service at the Stazione Zoologica Anton Dohrn di Napoli (Italy) is launched.

KEY WORDS

Taxonomy; Crustacea Amphipoda; network; MOTax; Italy.

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Taxonomy is today facing a variety of problems and a possible decline (Coleman, 2015) while at the same time, due to a possible loss of biodiversity predicted for the next years and the importance that the species have in this frame (Boero, 2010), an increasing interest is observed at different levels. Therefore, taxonomy has now a prominent role in all projects for the protection, preservation and restoration of the marine environment.

The Stazione Zoologica Anton Dohrn di Napoli (SZN), boasting a long tradition in taxonomic studies, has over time nurtured the idea to create a study area devoted to different aspects of taxonomy and in particular an Italian reference platform for marine taxonomy. This led to the creation of MOTax (Marine Organisms Taxonomy) (Fig. 1), which aims to promote a wide range of taxonomy initiatives and provide specialized identification



Figure 1. The MOTax logo: artistic creation by the colleague Flora Palumbo.

services based on integrated morphological and molecular approaches. MOTax will be organized in the frame of the European Marine Biological Resource Centre (EMBRC), a distributed European infrastructure which offers a range of high-level services

to support marine biology and ecology research, and in particular in the frame of the EMBRC-Italy, coordinated by the SZN. The President of the SZN R. Danovaro and the President of the SZN Scientific Council F. Boero strongly supported the birth of MOTax, affirming the need to enhance and share at national level the existing skills in marine organisms taxonomy and systematics. With the contribution of many SZN internal and external scientists, MOTax started its activity during the First Workshop MOTax “Marine Organisms Taxonomy: creation of a national integrated service in support of the EMBRC infrastructure”, which took place in Naples on 26 and 27 October 2016.

The main objectives of MOTax are:

- to coordinate the taxonomic expertise present in Italy, promoting the creation of a network of marine taxonomists, in the framework of the study and conservation of marine biodiversity;
- to create and optimize a national reference point in Europe providing taxonomic services and skills to research communities, through the EMBRC infrastructure;
- to define shared strategies aiming at developing a high-quality training in taxonomy.

To date the actions of MOTax have been:

- the “Naples Declaration” in which the taxonomist community expressed its position concerning the application of the Nagoya Protocol for marine genetic resources; the declaration was shared and signed by the experts who attended the Workshop and sent to the Italian Governmental Institutions;
- the creation of the “MOTax Newsletter”, aimed at sharing news, comments and other information concerning the taxonomy activity; issues are available at www.szn.it or can be requested to motax@szn.it;
- the launch of the “MOTax Survey”, the first national census to identify a national reference community for the systematics and taxonomy of marine organisms; the survey was distributed online to about 130 Italian taxonomists, mainly represented by the experts who attended the MOTax WS 2016.

Crustacean amphipods are one of the most represented taxa in marine and fresh waters, colonizing almost all habitats and systems. The Gulf of Naples and the Stazione Zoologica Anton Dohrn have a long tradition in studies on amphipods, it is enough to remember those of Costa (1853–7), Mayer (1882,

1890), Della Valle (1893), Cecchini & Parenzan (1934), Schiecke (1973) (see Ruffo, 1982-1998). Actually, in Italy amphipod studies meet at different levels the “true taxonomy”, therefore probably this is the right time to launch the proposal for an Italian amphipod “Working/Reference Group” or “Network” in the framework of MOTax, which may contribute to enhance and support taxonomy through several actions.

Important initiatives were carried out worldwide in the last years, such as the creation of the World Amphipoda Database (<http://www.marinespecies.org/amphipoda>) by T. Horton et al., which is part of the World Register of Marine Species (WoRMS), apart from a compilation of amphipod relevant literature in pdf files which may be downloaded from a Bibliography Server (<ftp://amphipod.dnsalias.net>) by C.O. Coleman, and the editing of Amphipod Newsletter, by W. Vader together with A. Baldinger, M. Lowe and A.H. Tandberg, which from 1972 provides lists of the new taxa, literature updating and news from the amphipodologists’ world. Furthermore, skills and outcomes are usually revealed and shown at the International Colloquium on Amphipoda (ICA), this year at the 17th edition.

In the Mediterranean Sea and in Italy, the important presence of S. Ruffo brought to the volumes of the Amphipoda of the Mediterranean (1982-1998) and to the checklist of the coasts of Italy (2010). After the loss of S. Ruffo the Italian experts on amphipods (and not only them) probably feel without a reference point, although the Museo Civico di Storia Naturale in Verona still hosts Ruffo’s precious amphipods collection, available for consultation (Ruffo & Krapp, 2005). Everyone certainly has the impression that nobody will substitute, in the future, such a great and special Person, but all together might probably contribute to avoid the dispersion of the knowledge achieved and stimulate amphipod studies in Italy.

As a proposal, the principal aims of the future Network should be focused on the following main actions:

- census of Italian amphipodologists, aiming at evaluating the state of art of knowledge; first of all to learn more about which are the approaches used in studying amphipods (Systematics, morphological and genetic taxonomy, genetics, population dynamics, structural and functional ecology, ethology, biodiversity, environmental stress, alien species,

biogeography), and where and in which habitat studies are being carried out, to facilitate exchange of information and collaborations;

- identification and promotion of research approaches on subject matters not yet addressed and on geographical areas not yet studied as concerns amphipod fauna;

- dissemination of scientific knowledge and promotion of amphipod studies among students and young researchers through short courses, seminars, exhibitions, etc., bringing the results under the Citizen Science;

- contribution to the constant updating of the Checklist of the Italian Amphipod Fauna;

- location and identification, also through available checklists, of amphipod collections present in both Museums (Innocenti, 2017) and Laboratories (personal collections) in Italy, and where they are available for consultation;

- archive in pdf format (eventually also in paper form) of the scientific production on amphipod studies conducted in Italy, which should include also the so called “gray literature”.

The network might include different levels of expertise, and the members will find it useful to exchange ideas and identify the major needs. Although constituted by Italian scientists, it could take advantage from the experience and cooperation of non-Italian amphipodologists who extensively worked along the Italian coasts and gave a fundamental contribution to the Italian amphipod fauna.

On the whole, the proposed actions seem very ambitious, but a first step towards this direction is desirable and feasible. Every expert on amphipods probably in the framework of her/his studies felt the necessity of at least one of the above mentioned actions! So if each of them puts some energy in what she/he believes more in, something good for all of us might be created.

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A first snapshot of sandy-beach amphipod (Crustacea) assemblage in a Marine Protected Area, Favignana Island (central Mediterranean Sea)

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ABSTRACT

The aim of this study is to compile a preliminary first check-list of Amphipoda species from beaches of Favignana Island (Sicily, Italy), and contribute to the knowledge relating to the distribution of this taxon in the Mediterranean Sea. Five amphipod species, belonging to two families (Talitridae and Hyalidae), have been collected in the island. The supralittoral assemblage appears to contain three main biogeographical categories: Atlanto-Mediterranean species, Mediterranean endemic species and cosmopolitan species.

KEY WORDS

Crustacea; Amphipoda; sandy beaches; Favignana Island; Mediterranean Sea.

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INTRODUCTION

To date no survey regarding the amphipod fauna of the coastal zone of Favignana Island (the largest of the islands Egadi Islands Marine Reserve in Sicily, Italy) has been conducted. This is notwithstanding that Marine Protected Areas (MPA) and marine reserves have been widely advocated as a form of marine conservation for preserving biodiversity (Agardy, 1994; Leslie, 2005).

This study will focus on the sandy beaches of Favignana Island, which are subjected to biotic and abiotic disturbances, and represented by natural and artificial bioturbation, mostly caused by recreational seashore activities. There is a growing interest in assessing factors and processes occurring in sandy beaches of Favignana Island (Bacci et al., 2015),

thus, an evaluation of amphipod assemblage need.

Sandy beaches are supralittoral areas, representing highly dynamic ecosystems that provide habitats for a diversity of fauna (Defeo et al., 2009). Due to the high vulnerability of these habitats, action plans for their conservation should be a priority; choosing a reliable target of organisms as an indicator for making decisions addressed to the preservation of biological diversity can be considered a fundamental task (Deidun et al., 2009; Lo Brutto et al., 2011; 2013; Sarà et al., 2014).

This paper will outline an initial assessment of the supralittoral Amphipoda assemblage, which are present along the sandy coasts of Favignana Island; the aim of the paper is in order to deepen our taxonomic knowledge regarding the composition of these species.

MATERIAL AND METHODS

The study was carried out on Favignana Island, which is located approximately five kilometers off the west coast of Sicily. Samples were collected during July - August 2015 from eight sites (37.93° N 12.27° E; 37.94° N 12.28° E; 37.93° N 12.32° E; 37.93° N 12.34° E; 31.94° N 12.36° E; 37.91° N 12.33° E; 37.91° N 12.32° E; 37.91° N 12.32° E), located in the northern and southern side of Favignana Island. These supralittoral sites, displaying two types of habitat, i.e. banquette of *Posidonia oceanica* (Linnaeus) Delile, 1813 or sand and algal/seagrass wrack, were sampled by means pitfall-traps: these consisted of plastic cups about 10 cm in diameter and 20 cm in height, positioned in the sand or in the banquettes. The traps were deployed at nightfall, at approximately 7.30 pm, and emptied at dawn in order to intercept individuals moving across the supra-littoral zone.

Identification of the collected specimens was carried out according to the morphological characteristics described by Ruffo ed. (1993), Iaciofano & Lo Brutto (2016; 2017) and Lowry & Fanini (2013).

RESULTS

On the whole 171 individuals of amphipods were collected: four of Talitridae, three belonging to the *Orchestia* Leach, 1814 genus, one species of the *Platorchestia* Bousfield, 1982 genus, and one species of Hyalidae, the latter belonging to the *Parhyale* Stebbing, 1897 genus.

This first checklist led us to document the occurrence of Amphipoda on Favignana Island. The supralittoral assemblage appears to contain three main biogeographical categories: Atlanto-Mediterranean species, *Parhyale aquilina* (Costa, 1857) and *Orchestia mediterranea* Costa, 1853, species endemic for the Mediterranean Sea, *O. montagui* Audouin, 1826 and *O. stephensi* Cecchini 1928, and cosmopolitan species, *Platorchestia platensis* (Krøyer, 1845).

In the Mediterranean Sea, talitrid family divided into three ecological categories: driftwood-hoppers: e.g. *Macarorchestia* spp., sand-hopper: e.g. *Talitrus saltator* (Montagu, 1808), beach-hopper: e.g. *Orchestia s.l.* (Lowry & Fanini, 2013).

In this study only the beach-hopper group was captured, though previous surveys collected both sand-hopper and beach-hopper categories in close geographic areas. Jelassi et al. (2015) recorded eight species of sand-hoppers and beach-hopper talitrids along Tunisian coastal lagoons. Prato et al. (1995) reported seven species, belonging to sand-hopper and beach-hopper groups, along the coast of Taranto (Ionian Sea, southern Italy); while Lowry & Fanini (2013) listed four species of sand-hopper and beach-hopper talitrids on the coast of Crete.

The sampling method (pitfall traps) may have selected species having mainly surface activity, i.e. beach-hopper, though Fanini & Lowry (2016) demonstrated that recreational use of the beaches can affect talitrid composition. In fact, sand-hoppers (substrate modifiers) appeared to be more sensitive than beach-hoppers (non-substrate modifiers) to such kind of bioturbation (Fanini & Lowry, 2016).

BRIEF GUIDE FOR BEACH-HOPPER IDENTIFICATION IN FAVIGNANA ISLANDS

Here a dichotomous key to species identification is presented.

- 1) A1 longer than peduncle of A2, U3 inner ramus poorly defined _____ *Parhyale aquilina*
A1 shorter or equal to peduncle of A2, U3 uniramous _____ 2
- 2) A2 peduncle inflated _____ *Platorchestia platensis*
A2 peduncle not inflated _____ 3
- 3) U1 peduncular spine-like seta absent, P5-P7 dactylus with a long seta on anterior margin _____ *Orchestia mediterranea*
U1 peduncular spine-like seta present _____ 4
- 4) P5-P7 dactylus with a long seta on anterior margin _____ *O. stephensi*
P5-P7 dactylus with a short seta on anterior margin _____ *O. montagui*

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Anthropogenic influence can affect the coexistence patterns of native gammarid species

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ABSTRACT

We examined how two native co-occurring gammarids (*Gammarus fossarum* Koch, 1836 and *G. roeselii* Gervais, 1835) are distributed among microhabitats, depending on their sympatric or allopatric distribution. Under near pristine circumstances, in cases of single species occurrences, no microhabitat preference was found in *G. fossarum*, whereas *G. roeselii* preferred lithal and biotic microhabitats. The presence of a competitor significantly modified the microdistribution: *G. roeselii* retreated from lithal microhabitats and *G. fossarum* almost disappeared from gravel. Due to anthropogenic impact the coexistence pattern of the two species modified in headwaters, resembling the pattern we found in the middle sections of streams.

KEY WORDS

Microhabitat preference; co-occurrence; habitat degradation.

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INTRODUCTION

Habitat segregation is considered an important process in forming and maintaining biodiversity (Leibold & McPeck, 2006). Antagonistic interactions among species belonging to the same functional guilds are generally based on predation and competition (Dick & Platvoet, 1996). Utilizing the same resources, such as food or habitat, may increase the probability of competition among similar species. Changes in native amphipod assemblages due to the colonization of invasive species are well-known (e.g. Grabowski et al., 2006). On the other hand, we found that the biotic interactions and habitat separation among native species were poorly known (Mauchart et al., 2014; 2017). To fill this gap we hypothesized that (H1) as closely-related species, the distribution of each species is equal among the available microhabitats in the absence of competitor, but (H2) in the case of co-existence,

due to the interspecific competition the microscale spatial distribution among microhabitats will change, and (H3) in case of strong anthropogenic influence and disturbance, this co-existence may exist under unusual circumstances.

MATERIAL AND METHODS

We sampled stream sections in South Transdanubia (SW Hungary) in 2009 according to AQEM protocol, while the most important local scale environmental variables were also measured. To identify the seasonal shifts in microhabitat preferences, Kruskal-Wallis tests were used.

RESULTS AND CONCLUSIONS

Testing H1 and H2 we found that under natural

conditions the gammarid species showed different microhabitat preferences at sites where they co-occurred (middle stream section), compared to the microhabitat choice of single occurrences (upper and lower sections, separately, Fig. 1.). Till *Gammarus roeselii* Gervais, 1835 had a definite microhabitat preference, *G. fossarum* Koch, 1836 showed preference only if both species were co-occurred. In those cases, *G. fossarum* was mostly abundant in the lithal and biotic microhabitats, whereas it was less dominant in gravel ones. On the contrary, *G. roeselii* was almost completely absent from the lithal microhabitats, but it was usually frequent in

biotic microhabitats. According to H3, we observed that in case of stronger anthropogenic impacts (e.g. highly degraded riparian vegetation) *G. roeselii* appeared at disturbed headwater sites with similar co-existence pattern which was found under natural circumstances in the middle section of the streams. We conclude that the degradation of near-pristine headwater sites could enable *G. roeselii* to further expand its area of occurrence and to occupy sections which basically, without disturbance, are not suitable for them.

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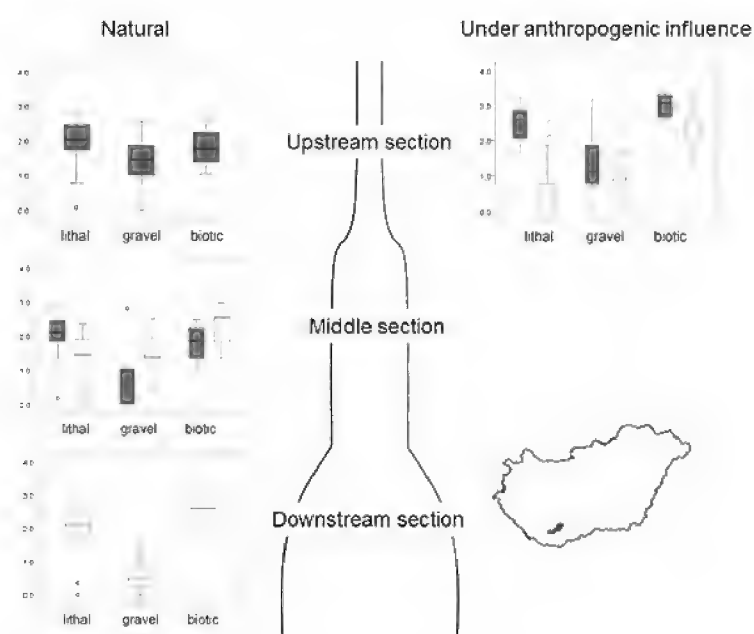


Figure 1. The microhabitat preference of the gammarid species depending on the longitudinal section and disturbance of streams. Solid boxes are the $\log_{10}(x+1)$ transformed abundance of *G. fossarum*; empty boxes are that of *G. roeselii*. (□: interquartile range; —: median; T: minimum and maximum values without outliers and extremes; ○: outlier; *: extreme).

Updating taxonomic practice to cope with challenges from within and without the discipline

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ABSTRACT

Two contentious issues are examined, that are increasingly troubling taxonomy. One is the increasingly frequent lack of formal description and naming of species whose existence is hypothesized based on molecular evidence, coupled with the undisciplined usage of formulae to label either hypothesized species taxa, or inadequately identified samples, in a way that defies future comparison with other samples or species. The second issue is the increasing appreciation of the commonly directional and occasionally saltational evolvability of characters, morphological and molecular alike - a stimulating but hitherto poorly exploited contribution of evolutionary developmental biology to taxonomy.

KEY WORDS

Cryptic species; evolvability; evo-devo and taxonomy; nomenclature; sequence data bases

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Joel Hedgpeth (1911–2006), authoritative marine biologist, environmentalist and pycnogonid specialist, devoted a famous lecture (Hedgpeth, 1962) to man's oldest profession. If you are not sure about the identity of this profession, I will immediately reassure you: that lecture's subject matter was taxonomy, the discipline practiced by Adam when he named the creatures of the Garden of Eden, today a precious companion to everybody in biology, pure and applied alike. It is also on taxonomy that I will focus here, to briefly discuss two contentious issues that are increasingly troubling its practitioners. The roots of the first problem mainly are intrinsic to the discipline, while those of the other emerge out of a confrontation with disciplines with which taxonomy cannot avoid to have intercourse.

But let's begin from outside taxonomy, that is, from the needs and expectations of the variegated community of taxonomy's users. This community includes two main components. On the one side,

scientists - from experimental biologists to ecologists to researchers in stratigraphic geology, who need clear-cut criteria for species identification, a solid and possibly unchanging nomenclature and an acceptable and basically stable framework of supra-specific classification. On the other side, people like lawyers who write, or try to apply, sets of rules for nature conservation - people who need to refer with any possible degree of certainty to unequivocally identifiable kinds or organisms designed by universally accepted names. Despite its sheer heterogeneity, this community is tightly unified by a common need for stability, taxonomic and nomenclatural alike.

At the opposite end of the spectrum there is an increasingly large number of evolutionary biologists who delight in controversial taxa that do not find a stable place into the hierarchy of Linnaean taxonomy and even challenge in principle the possibility to arrive at an acceptable agreement on the notion of species (cf. Zachos, 2016), and do not care

for the unavoidable consequences of this turmoil on the general application of Linnaean nomenclature. A number of philosophers of biology has joined this camp and contributes to endless discussions on the nature of species and the Linnaean hierarchy.

What about the professional taxonomist? For those whose academic profession is man's oldest, life is becoming more and more difficult. This is not due to shortage of partners: a coarse but unambiguous indicator of taxonomy's wellness is the positive trend in the number of yearly described species (International Institute for Species Exploration, 2011, 2012). Problems, instead, are due to two opposite pressures: one, more obvious, from within, in the form of increasing occurrence of approaches to describing and naming biodiversity alternative to the traditional ones; the other from without, urging to reformulate some concepts and practices in taxonomy, to keep apace with current progress in other biological disciplines. I will mention here two of these critical areas of taxonomy, and provide suggestions on how to steer the boat towards intellectually and operationally profitable alternatives.

NEW TAXONOMIC APPROACHES MAY LEAD TO GREY NOMENCLATURE

In an increasing number of articles in which the hypothesis of the occurrence of cryptic species-level taxa is formulated, these are not described in full and named according to the Code (cf. Minelli, 2015). Among the examples discussed in Minelli (2017a) one is about amphipods: none of the 33 "provisional species" recognized by Witt et al. (2006) within a clade of freshwater amphipods from desert springs of the southern Great Basin of California and Nevada, USA, all hitherto referred to the one species, *Hyaella azteca* (Saussure, 1858), has been described and named to date. For the time being, the only way to refer to them is by using haplotypes acronyms/formulae (e.g., HaPS23) while the only descriptive detail available about them are the sequence data available in Barcode of Life Data Systems. However, we may hope that these species will be eventually named and described formally, as repeatedly advocated for cryptic species generally (e.g., Pérez-Ponce de León & Nadler, 2010), and for those of amphipods in particular (e.g., Trontelj & Fišer, 2009). Different and much less tractable problems are caused by the names of

taxonomic units in sequence databases and the associated, sequence-based taxonomies.

In the largest sequence databases, in particular those of the Barcode of Life Data Systems (BOLD; <http://www.barcodinglife.org/>) and GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>), both formal Linnaean names and informal names and formulae are used to identify specimens, operational units and formal taxa. The main problem with this diverse set of labels is not the fact that many of them do not follow the precepts of the Code, but the fact that most of these terms follow no standard at all, and are often ambiguous, or silent, about the kind of information they are intended to carry. These labels form an inconvenient grey nomenclature.

As a sample of the taxonomic terms used in BOLD, I analyzed the taxonomic list for the Amphipoda. As of 27 June 2017, the 'species list' for this group contains 1250 Linnaean binomials, plus 555 entries identified only at genus (in a few cases, just family) level, another 123 only tentatively referred to a named species, alongside a great number of formulae devoid of any nomenclatural significance, such as *Austrochiltonia* sp. Clade A4c, AoridaeNZD (sic) sp. 02, *Niphargus* cf. *stygius*, AH_10.12, *Leucothoe kensleyi* 10VPR, *Synurella* sp. 2 AG2014.

Admittedly, remarkable progress with the unique identification of sequence-based taxonomic units (for a rationale, cf. Tautz et al., 2003; Ratnasingham & Hebert, 2013) was obtained by introducing in the BOLD database the Barcode Index Numbers (BINs), arguably the best example of how a non-Linnaean nomenclature can be planned and used. BINs are partitions of the total diversity of the sequences deposited with the BOLD database generated dynamically, that is, continuously changing, following the addition of new records in the database. It must be kept in mind that these alphanumerical codes are not intended as substitutes for species names. Moreover, sequences from specimens currently referred to two different species are frequently clustered in the same BIN, although this does not necessarily mean that those species should be regarded as synonymous. On the other hand, sequences from specimens identified as belonging to the same species are often clustered in more than one BIN. This is the case, for example, of the amphipods currently known under the Linnaean name of *Hyaella azteca*, mentioned before.

BINs are an excellent example of how to create and use non-Linnaean formulae for segments of biological diversity, because their meaning is unequivocally defined. These qualities are sadly lacking in the other kinds of grey nomenclature mentioned above. There are here three main problems.

First, the lack of rules for the creation and usage of these names of formulae precludes unambiguous understanding of what the name or formula is for. For example, what does “*Niphargus* sp. 32” mean? We have no standard of reference (not to mention a code) to which to refer.

Second, it is often unclear, especially in database entries, if the alphanumeric specifiers added to a genus name, or even to a Linnaean binomen, are intended to provide unambiguous labelling for a single specimen, a taxon, or both.

Third, and most important, because of the subjectivity of the criteria according to which these non-Linnaean names are created and used, it is generally impossible to compare them across studies or databases.

It would be nonsense to fight against the use of these non-Linnaean names and formulae. But this grey nomenclature needs a small set of simple, clear principles, fixing the standard format for each of the objects we need to distinguish. In a recent paper (Minelli, 2017a) I suggested a few principles upon which we should establish a small set of rules for this non-Linnaean nomenclature:

- definition of a number of standard formats for the different kinds of objects (or hypotheses) we need to label, such as individual species, undescribed new species, or other
- preservation (and exhaustive labelling) of material vouchers, including both whole specimens and DNA sequences
- permanent association of the “grey name” with source information such as author and year for names introduced in a publication, or equivalent information, in suitable format to be specified, for unpublished database entries.

EVOLVABILITY AND CHARACTER POLARITY

In cladistics, character polarity is established based on a number of criteria (e.g., outgroup comparison) through which the information provided by

the distribution of character states in relevant (ingroup and outgroup) taxa, sometimes also in different semaphoronts (e.g., ontogenetic stages) is extracted. It is only at the level of nucleotide sequences that the a priori probabilities of different kinds of change (essentially, transitions vs. transversion) were taken into account, as suggesting different amounts of phylogenetic signal; admittedly, however, if and how to translate this appreciation into differential weighting of transversions and transitions was soon acknowledged to be a seriously contentious matter (see e.g. Broughton et al., 2000; Kjer et al., 2007).

Biased polarity of change is, however, a pervasive feature of evolution, affecting all kinds of traits, morphological as well as molecular. Recent developments in evolutionary developmental biology (evo-devo) are revealing indeed peculiar scenarios of evolvability (e.g., Arthur, 2004; Minelli, 2017b), in the light of which character coding should be thoroughly revisited (Minelli, 2015b).

Moreover, in the light of evo-devo it becomes possible to accept that characters may occasionally evolve in a saltational way. Accordingly, a major morphological difference in a usually conservative trait should not deter from assuming close relationship between two otherwise similar species and occasional teratological specimen may open a precious window into otherwise poorly accessible scenarios of evolvability. As for amphipods, the mouthparts of *Haploginglymus morenoi* Iannilli, Minelli et Ruffo, 2009 its maxilliped in particular, are unmatched in the whole of the Amphipoda, however, by describing this taxon, the authors (Iannilli et al., 2009) preferred not to create a new genus for it, because the anomalous condition found in one of the specimens of the type series, where the left part of the maxilliped showed a dactylus provided with a normal claw, whereas the right one was transformed into the spoon-like paddle characteristic of the species, suggested easy transformation between the two character states.

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The diversity and antibiotic properties of actinobacteria associated with endemic deepwater amphipods of lake Baikal

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ABSTRACT

In this study we isolated 42 actinobacteria strains from crustaceans belonging to *Ommatogammarus albinus* (Dybowsky, 1874) and *O. flavus* (Dybowsky, 1874). This is the first report describing the isolation and initial characterization of representatives of *Micromonospora* and *Pseudonocardia* genera from Baikal deepwater invertebrates. More than 70 % of isolated strains demonstrated antifungal activity. The dereplication analysis of extract of one of the isolated strains resulted in annotation of several known compounds that can help to explain the observed biological activities. The characteristics of ecological niche and lifestyle of deepwater amphipods suggests that the observed associations between crustaceans and isolated actinobacteria are not random and might represent long-term symbiotic interactions

KEY WORDS

Actinobacteria; amphipods; Baikal; *Ommatogammarus* spp.; endemics.

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Natural products remain the most important source of new pharmaceutical leads, including antibiotics (Doroghazi et al., 2014). The multiple reports on emergence of pathogenic bacteria resistant to antibiotics rise the need for effective antimicrobials with new modes of action (Ling et al., 2015). The uniqueness of biotic and abiotic factors makes Lake Baikal and its inhabitants a promising source of new actinobacteria species producing novel biologically active compounds with chemically and pharmaceutically interesting properties. The present study aims to examine cultivated actinobacteria population associated with Lake Baikal endemic deepwater amphipods for the antibiotic activity accompanied with the dereplication of secondary metabolites produced by some of them.

Two hundred specimens of two endemic deepwater amphipod species of the genus *Ommatogammarus* Stebbing, 1899, *O. albinus* (Dybowsky, 1874) and *O. flavus* (Dybowsky, 1874), were collected at different depths of 80 m, 100 m and 200 m. The amphipods were captured in traps baited with sterilized putrescent fish. Immediately after the lifting the traps, the amphipods were rinsed with 70% ethanol following by sterile water (three times) to remove transient bacteria and then homogenized in sterile glycerol. The actinobacteria strains were isolated by plating obtained homogenate on solid nutrient media. Herein, we report isolation and initial characterization of 42 strains of actinobacteria from Lake Baikal endemic deepwater amphipod species *O. albinus* and *O. flavus*. Among them, 40

were identified as representatives of genus *Streptomyces* and two belongs to minor genera of *Micromonospora* and *Pseudonocardia*. This finding correlates with the previous observations of phylogenetic diversity of microbial communities of Lake Baikal water and sediments.

Based on the obtained materials, some correlations could be observed between the number of actinobacteria isolates, the depth of sampling and the species of amphipod used as a source. For example, the number of actinobacteria decreased in the case of *O. albinus* along the depth gradient from 80 (n=11) to 200 m (n=1). In the case of *O. flavus* the opposite trend was observed: the number of actinobacteria strains increased with the depth. The deeper understanding of ecology of the studied amphipods can help to explain the observed phenomena. Both species are known for the seasonal migration activities (from depth of 2.5 m to 1313 m in the case of *O. flavus* and from depth of 100 m to 1600 m in the case of *O. albinus*) (Timoshkin, 2001). These migrations are caused by factors related to feeding and probably can influence the microbial associations of amphipods depending on the substrates exploited.

We also found that large proportion (more than 70%) of strains isolated from amphipods have anti-fungal activity. This makes us to believe that these associations are not random and could represent symbiotic interactions between bacteria and amphipods. The amphipods consume the organic matter and detritus which are naturally inhabited by fungi as decomposers (Su et al., 2015).

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Amino acid and isotopic profile of two invasive gammarids, *Dikerogammarus villosus* (Sowinsky, 1894) and *Gammarus roeselii* Gervais, 1835, from Lake Constance

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ABSTRACT

The stable isotopes, carbon ($\delta^{13}\text{C}$) and ($\delta^{15}\text{N}$), to test the hypothesis that dietary segregation in cohabiting invasive gammarids were used.

KEY WORDS

Stable isotopes; amino acids.

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Among the mechanisms that allow competing species to coexist are resource partitioning and dietary segregation. The current study uses dual stable isotopes, carbon ($\delta^{13}\text{C}$) and ($\delta^{15}\text{N}$) to test the hypothesis that dietary segregation in cohabiting invasive gammarids, *Dikerogammarus villosus* (Sowinsky, 1894) and *Gammarus roeselii* Gervais, 1835, will be reflected by differences in isotope values. Furthermore, IsoError mixing models were used to estimate the relative contributions of periphyton and seston to the invaders' diets. Whole tissue $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis using samples obtained in autumn/winter in the *D. villosus* ('killer shrimp') and *G. roeselii* exhibited no significant difference in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, indicating a considerable overlap

between the dietary sources of these sympatric invaders. Furthermore, we used essential and non-essential amino acids (Alanine, Asparagine, Glutamine, Glycine, Leucine, Lysine, Methionine, Phenylalanine, Proline and Valine) specific $\delta^{13}\text{C}$ analysis and found no significant difference in any of the amino acids explored. These results indicate a lack of a clear feeding niche differentiation in the invasive gammarids. These data suggest that the cohabitation characteristics of the two invasive species coupled with their voracious behavior could in fact assist their success at co-invasion. This might have serious implications for local biodiversity including the potential extinction of native species.

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Impact of Arbuscular Mycorrhizal Fungi and Fertilization Levels on biochemical changes in potato (*Solanum tuberosum* L., Solanales Solanaceae)

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ABSTRACT

Experiments have been carried out to evaluate the potential biochemical mycorrhizal benefit of potato (*Solanum tuberosum* L., Solanales Solanaceae) grown in northeastern Algeria. Three doses of chemical fertilizer (NPK) corresponding to 0, 50 and 100% of the recommended dose have been applied in presence or not in presence of the mycorrhizal inoculant. The results have revealed that the additional contribution of arbuscular mycorrhizal fungi combined with 50% chemical fertilizer gives the best results. A significant increase has been observed at the root colonization and chlorophyll content level. In terms of tuber quality, there is a significant increase in starch and protein content. However, the application of mycorrhizae alone will not compete with synthetic fertilizers in vegetable gardening, but could reduce their applications and thus improve yield while preserving the environment.

KEY WORDS

Commercial AMF; Biochemical contents; Potato; Fertilization; vegetable crops.

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INTRODUCTION

The potato (*Solanum tuberosum* L., Solanales Solanaceae) is the most important tuber crop in the world, grown in more than 125 countries and consumed almost daily by more than 1 billion people (FAO, 2013) and is among the main vegetal products which can make it possible to combat poverty in the world (Lutaladio & Prakash, 2010).

It is one of the most demanding crops of fertilizers, due to its shallow and less developed root system that makes its area of exploration and nutrient mining limited. Potato crops need adequate strategies for disease control and fertilization to maximize yield. All of these chemicals could have

adverse effects on the environment and health. There is a growing need for integrated potato cropping systems that combine different management strategies with reduced use of mineral fertilizers and pesticides (Maynard & Hochmuth, 2007).

According to the researchers, the shift towards more productive and less phosphate-based chemical inputs cannot be achieved without better management of biological interactions in agroecosystems, such as mycorrhizae (Plenchette et al., 2005; Adesemoye & Kloepper, 2009). They are root-associated symbiotics and show strong growth-promoting effects for most plants, including almost all edible crops, by increasing the contribution of available phosphorus from soil (P) and other nutrients. Im-

mobile minerals essential for plant growth, they are key players in the sustainable management of agricultural systems (Smith & Read, 2008). In several countries, inoculation practices have proved to be a very effective solution for increasing crop yields and reducing their need for phosphate mineral inputs (Hamel & Plenchette, 2007).

The use of arbuscular mycorrhizal fungi (AMF) to improve potato growth has been a focus of several studies (Hijri, 2006, Douds et al., 2007; Wu et al., 2013) which have shown the efficiency of these microorganisms for this culture. In Algeria, the potato sector in all its aspects of seeds and consumption now holds a strategic position in the new policy of agricultural and rural renewal, where its culture remains among the market gardening species which are fundamental for feeding strategy (FAO, 2015). It would be interesting to study the potential benefits of commercial mycorrhizal inoculants in this crop that could open up a very attractive market for these products.

In real field cropping conditions, the aim of this project is to determine the effect of mycorrhizal inoculation on certain biochemical parameters of potato plants for a better quality of tubers and to study the possibility of reducing the applications of chemical fertilizers for a better environment.

MATERIAL AND METHODS

Study area

The experiment has been carried out in a private agricultural field situated in the locality of Drean, Taref wilaya, Algeria (36°41'00"N, 7°45'00"E). The climate is of the Mediterranean type. The period of cultivation which runs from 13th January 2014 to 13th June 2014 is characterized by average maximum temperatures of 21 °C and minimum of 9 °C, total precipitation being 255 mm. The substrate chosen for the implementation of the experiment is a clayey soil having the preceding crop as wheat. These characteristics have been determined from a composite sample of 0-20 cm taken from deep soil. The physicochemical properties of the soil have been determined in the agricultural laboratory of FERTIAL (Annaba, Algeria): 28% sand, 44% clay, 24% silt; pH 7.1; 13.60 ppm available P;

0.76% total C; 0.99 meq / 100 g K; 35.35 meq / 100 g Ca; 5.22 meq / 100 g Mg; 0.87 meq / 100 g Na; 0.952% organic matter.

Vegetable material

The plant material consists of potato tubers (*Solanum tuberosum* L., Solanales Solanaceae), a Spunta variety fast-growing, giving large-sized tubers.

Fungal material

The fungal material used is a commercial mycorrhizal inoculum called Symbivit®, produced by a French company specialized in the development and distribution of mycorrhizogenic products (INOCULUM plus, France). It consists of a natural clay base and propagules of 6 species AMF (*Claroideoglomus etunicatum*, *C. claroideum*, *Glomus microaggregatum*, *Rhizophagus intraradices*, *Funneliformis mosseae*, *F. geosporum*).

Experimental device and processing of treatments

The complete random block device has been used, it comprises three blocks corresponding to the number of repetition, each block contains all the treatments, the distribution of the treatments within the same block was made randomly by drawing by lot. In a block there are six associated plots in pairs, each consisting of 4 rows which are 75 cm apart and six rows with a spacing of 20 cm between the plants. The surface area of each plot was 2.25 m² (2.2 m wide and 1 m long).

The treatments that have been applied are:

- inoculation with Symbivit and not (control) on plots without performing chemical contribution (0% NPK)
- inoculation and not on plots having been enriched in doses of localized fertilizers (50% NPK) corresponding to 30 g / plant
- inoculation and not with 100% (NPK), ie 60 g/plant.

The quantities of mineral and organic fertilizers introduced during the trial recommended in the Dréan area for potato production are: nitrogen in the form of ammonium nitrate (N33%:100 kg/ ha⁻¹) Phosphorus in the form of triple superphosphate

(P_2O_5 45%: 150 kg/ ha $^{-1}$) and potassium in the form of K_2SO_4 sulphate (K_2O 54%: 400 kg/ ha $^{-1}$).

Data collection

After three months of cultivation in the vegetative phase, 5 plants have been selected from each plot and the concentration of chlorophyll has been measured with a SPAD meters.

The rate of root colonization has been estimated and staining has been done by the technique of Phillips & Haymann (1970). The root samples are mixed and placed in 10% KOH solution, in a Marie bath at 90 °C for 1 hour, then rinsed and stained with 0.05% Trypan blue solution for 15 minutes. The roots have been observed on 5 replicates of root fragments of 1 cm length mounted between the blade and the slide in a drop of glycerol. The annotation has been made according to the method described by Trouvelot et al. (1986), using the MYCOCALC software (www.dijon.inra.fr/mychintec/Mycocalc-prg/download.html).

The protein content of the potato tubers has been estimated after harvest using the technique of Lowry et al. (1951), the absorbance readings have been taken at 660 nm.

The total amount of starch present in the potato tubers has been estimated using the anthrone method proposed by Hedge & Hofreiter (1962). Absorbance readings have been taken at 630 nm.

Methods of data statistical analysis

The description of the different studied characteristics of the plant is made by calculating the mean (m), the standard deviation (s) and the minimum (Xmin) and maximum (Xmax) values for each treatment.

The variance analysis (ANOVA) to a classification criterion of the Minitab software for the data statistical analysis (Minitab Inc, 2014) was used to compare the averages of the three doses of the fertilizer (NPK) for each studied characteristic (Dagnelie, 2009).

The TUKEY test (Dagnelie, 2009) made it possible to determine the groups of homogeneous doses by plant characteristics (Minitab Inc, 2014).

The DUNNETT test (Dagnelie, 2009) was used to compare the mean of the control dose with each of the averages of the other doses, for each parameter of the plant (Minitab Inc, 2014).

The STUDENT T test compared the averages of the two treatments using data from two independent samples (Dagnelie, 2009).

RESULTS

Table 1 illustrates the values of the statistical parameters obtained through characteristics and per dose of the fertilizer according to treatment (inoculated/non-inoculated) for the plant. The mean values and the standard deviations are represented graphically by histograms in figures 1–4.

The results of the analysis of variance (ANOVA) are presented in Table 2, showing a highly significant effect on the chlorophyll content of the potato leaves between the averages of the three doses of the fertilizer, either for the inoculated and non-inoculated plants.

A very highly significant difference has been found for the starch and protein content of inoculated tubers, and highly significant for non-inoculated tubers. As for the root colonization rate, it shows a significant difference for mycorrhizal plants and not significant for non-mycorrhizal plants.

The TUKEY test, following the rejection of ANOVA's hypothesis of equality of means, shows that there are two groups of homogeneous doses for chlorophyll (leaves), starch and protein (tubers) content for the inoculated or non-inoculated plant. For the root colonization rate there is a single group for the mycorrhizal plants and two groups for the non-mycorrhizae. The alphabetical letters (a, b) for the inoculated plants and (a', b') for the uninoculated plants designate these groups (Figs. 1–4).

Table 3 shows the results of the DUNNETT test, which shows that the 50% and 100% fertilizer doses give different results from the 0% dose for chlorophyll, protein and starch content in inoculated plants. In the case of non-mycorrhizal plants, the 0% dose and the 50% fertilizer dose are the same for the chlorophyll and starch content, with respect to the protein content of no inoculated tubers, the samples from the 0% fertilizer dose are different from those from the 50% and 100%. The colonization rate for the three doses of the fertilizer is identical for the case of the inoculated plants. For non-inoculated plants, the D0 is different from the two other doses.

The results of the STUDENT *t* test show that the combination of the mycorrhizogenic inoculum with the various doses of chemical fertilizers has got variable effects on the studied biochemical parameters of the potato. In the absence of fertilizer (0% CF), the difference between the inoculated and non-inoculated plants, for the potato leaf chlorophyll content and the amount of protein in the tubers, is not significant. On the other hand, the starch content of the inoculated tubers increases significantly compared to non-inoculated controls. The mycorrhization of the plants with half the amount of the fertilizer D50 is sufficient to get very highly significant differences in chlorophyll leaf content and in the starch and protein concentration of the tubers, with an increase of about 37%, 32% and 27% respectively. The differences are also very highly significant between inoculated and non-inoculated plants for the full dose of D100 fertilizer.

Mycorrhizal inoculation significantly increases the rate of root colonization whatever the level of fertilization is. However, the treatment (I + 0% CF) shows the highest rate with 47%. The root colonization in uninoculated plants shows the lowest level with 100% CF (20%).

DISCUSSION

This research was carried out with the aim of studying the efficiency of mycorrhizal inoculation in the field on potato cultivation and to evaluate the potential of the commercial inoculum to reduce the inoculum fertilizers.

The results show that the chlorophyll content value is higher in the case of mycorrhizal plants, the treatment combining the inoculum with 50% of the recommended fertilizer dose gives good results, besides the plants that have not received a fertilizer supply have shown poor chlorophyll rate, probably due to nutrient deficiency in the soil (Soltner, 1981). The inoculated plants are more likely to produce food through photosynthesis. Ruiz et al. (1996), Wright et al. (1998) suggested that mycorrhizal colonization helps the absorption of inorganic nutrients. Eissenstat et al. (1993) observed an increase in leaf area by arbuscular mycorrhizal fungi and therefore an increase in photosynthetic activity which explains an increase in chlorophyll content in the inoculated samples.

The protein content in the tubers has shown higher values in the mycorrhizal plants than in the controls. Our results are similar to the results obtained by Lenin et al. (2010) when studying the effect of arbuscular fungi on biochemical parameters and the growth evolution of four different vegetable crops. The increasing levels of protein in inoculated plants could be due to either the presence of fungal proteins or infectious stimulation of protein synthesis in the host plant (Krishna & Bagyaraj, 1983). Mycorrhizal and non-mycorrhizal plants are recognizable by a difference in the biochemical constitution, in particular in the amino acid and protein fractions (Nemec & Meredith, 1981).

Starch is one of the main important biochemical components in a potato tuber. We have observed that the inoculated plants contain higher concentrations of starch, in particular those which have received 50% of fertilizer; Wu & Xia (2006) also obtained similar results when the amount of starch was observed to be higher in the mycorrhizal plants.

The root colonization rate was higher in the inoculated plants than in the controls, as a commercial inoculum contribution probably favored the development of the mycelial network related to the roots of the inoculated plants (Smith & Read, 2008). We have observed a decrease in colonization of non-inoculated roots, especially for the 50 and 100% doses. Al-Karaki (2013) showed that increasing soil fertilization may reduce colonization of roots AMF and spore density.

CONCLUSIONS

Observation of the data set reveals that the additional contribution of mycorrhizogenic arbuscular fungi taken as inoculum significantly improves the quality of the potato. Combined with the chemical fertilizer at 50% of recommended doses, this experience gives better results. However, mycorrhizae alone will not compete with chemical fertilizers. The best combination would therefore be to reduce the use of synthetic fertilizers and to provide an inoculum in addition to the mycorrhizal populations already existing in the soil. This suggests that the advised application for chemical fertilizers could possibly be reduced at least by half by applying mycorrhizal inoculum.

Treatment	variables	Doses (NPK)	n	m	s	X _{min} ____X _{max}
Inoculated	Chlorophyll	D ₀	15	40.360	6.127	33.670 ____ 55.650
		D ₅₀	15	56.868	3.029	46.650 ____ 57.300
		D ₁₀₀	15	59.999	4.494	45.720 ____ 56.890
	Proteins	D ₀	15	0.952	0.177	0.150 ____ 1.890
		D ₅₀	15	1.977	0.364	1.780 ____ 2.890
		D ₁₀₀	15	2.238	0.4982	2.470 ____ 2.960
	Starch	D ₀	15	13.673	1.65	10.580 ____ 16.890
		D ₅₀	15	18.738	2.551	14.360 ____ 19.650
		D ₁₀₀	15	20.110	3.012	16.480 ____ 20.740
	Root mycorrhization rate	D ₀	15	47.026	6.484	33.670 ____ 55.650
		D ₅₀	15	41.099	7.239	46.650 ____ 57.300
		D ₁₀₀	15	38.206	4.112	45.720 ____ 56.890
No inoculated	Chlorophyll	D ₀	15	31.144	7.329	29.890 ____ 50.100
		D ₅₀	15	35.670	7.256	23.760 ____ 46.780
		D ₁₀₀	15	42.327	7.107	29.800 ____ 49.980
	Proteins	D ₀	15	0.683	0.116	0.450 ____ 1.780
		D ₅₀	15	1.424	0.167	1.780 ____ 2.120
		D ₁₀₀	15	1.494	0.301	1.250 ____ 2.660
	Starch	D ₀	15	10.820	1,487	10.850 ____ 16.360
		D ₅₀	15	12.725	1.595	11.320 ____ 18.360
		D ₁₀₀	15	15.704	2.597	15.210 ____ 20.120
	Root mycorrhization rate	D ₀	15	29.216	3.206	18.250 ____
		D ₅₀	15	23.712	3.90	30.450
		D ₁₀₀	15	20.275	3.620	17.63 ____ 32.36
						16.23 ____
						30.960

Table1. The values of the basic statistical parameters calculated on potato plant samples; legenda: number of samples (n), mean (m), standard deviation (s), minimum values (Xmin), and maximum values (Xmax).

Treatment	variables	Source of variation	ddl	SCE	CM	F	P
Inoculated	Chlorophyll	doses	3	218.77	109.39	5.90	0.006**
	Proteins	doses	3	81.386	40.693	31.89	0.000***
	Starch	doses	3	160.814	80.407	28.70	0.000***
	Root mycorrhization rate	doses	3	37.39	18.69	0.26	0.023*
No inoculated	Chlorophyll	doses	3	112.88	56.14	1.26	0.007**
	Proteins	doses	3	181.456	90.728	42.58	0.003**
	Starch	doses	3	169.453	84.727	27.69	0.002**
	Root mycorrhization	doses	3	12.88	6.44	0.50	0.610 n.s

Table 2. Results of the analysis of variance (ANOVA) to a criterion. Legenda: number of degrees of freedom (ddl), sum of the squares of the deviations (SCE), mean square (CM), observed value of the Fisher F variable (Fobs.) and probability (P). N.S: there are no significant differences; *: there are significant differences ($p \leq \alpha = 0.05$); **: there are some highly significant differences ($p \leq \alpha = 0.01$); ***: there are very highly significant differences ($p \leq \alpha = 0.001$).

Treatment	variables	Means and doses identical to the control dose		
Inoculated	Chlorophyll	D ₀ <u>40.36</u>	D ₅₀ 56.86	D ₁₀₀ 59.99
	Proteins	D ₀ <u>0.952</u>	D ₅₀ 1.97	D ₁₀₀ 2.23
	starch	D ₀ <u>13.67</u>	D ₅₀ 18.73	D ₁₀₀ 20.11
	Root mycorrhization rate	D ₀ <u>47.02</u>	D ₅₀ <u>41.09</u>	D ₁₀₀ <u>38.20</u>
No inoculated	Chlorophyll	D ₀ <u>31.14</u>	D ₅₀ <u>35.67</u>	D ₁₀₀ 42.32
	Proteins	D ₀ <u>0.68</u>	D ₅₀ 1.42	D ₁₀₀ 1.49
	Starch	D ₀ <u>10.82</u>	D ₅₀ <u>12.72</u>	D ₁₀₀ 15.70
	Root mycorrhization rate	D ₀ <u>29.21</u>	D ₅₀ 23.71	D ₁₀₀ 20.27

Table 3. Results of the DUNNETT test. The averages of the underlined doses are identical to the mean of the control dose (D₀).

Treatment	variables	Dose averages and homogeneous groups			Number of groups
Inoculated	Chlorophyll	<u>D₀</u> 40.36	<u>D₅₀</u> 56.86	<u>D₁₀₀</u> 59.99	2
	Proteins	<u>D₀</u> 0.952	<u>D₅₀</u> 1.97	<u>D₁₀₀</u> 2.23	2
	Starch	<u>D₀</u> 13.67	<u>D₅₀</u> 18.73	<u>D₁₀₀</u> 20.11	2
	Root mycorrhization rate	<u>D₀</u> 47.02	<u>D₅₀</u> 41.09	<u>D₁₀₀</u> 38.20	1
No inoculated	Chlorophyll	<u>D₀</u> 31.14	<u>D₅₀</u> 35.67	<u>D₁₀₀</u> 42.32	2
	Proteins	<u>D₀</u> 0.68	<u>D₅₀</u> 1.42	<u>D₁₀₀</u> 1.49	2
	Starch	<u>D₀</u> 10.82	<u>D₅₀</u> 12.72	<u>D₁₀₀</u> 15.70	2
	Root mycorrhization rate	<u>D₀</u> 29.21	<u>D₅₀</u> 23.71	<u>D₁₀₀</u> 20.27	2

Table 4. Result of the TUKEY test. Search for homogeneous dose groups.

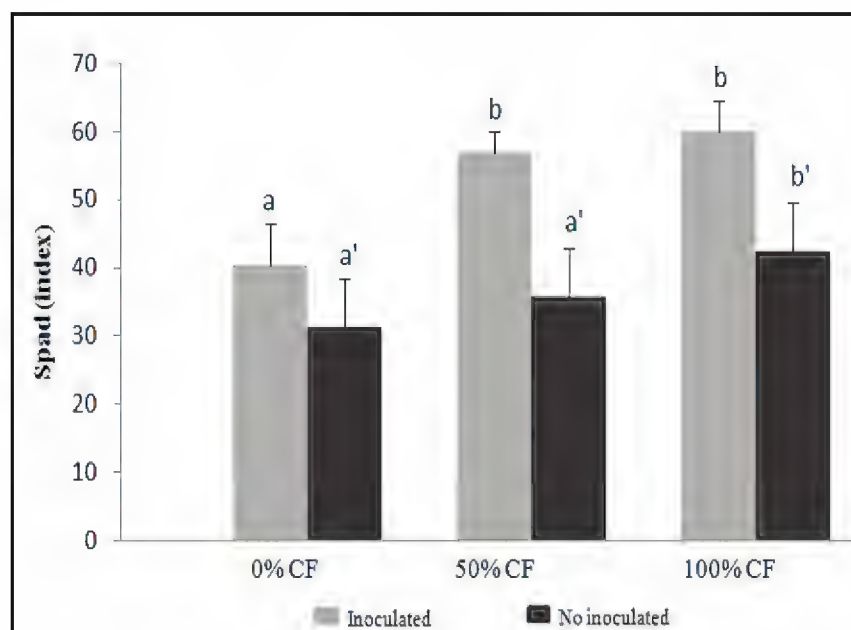


Figure 1. Chlorophyll content of potato leaves in the presence of three doses of NPK fertilizer for inoculated and non-inoculated plants.

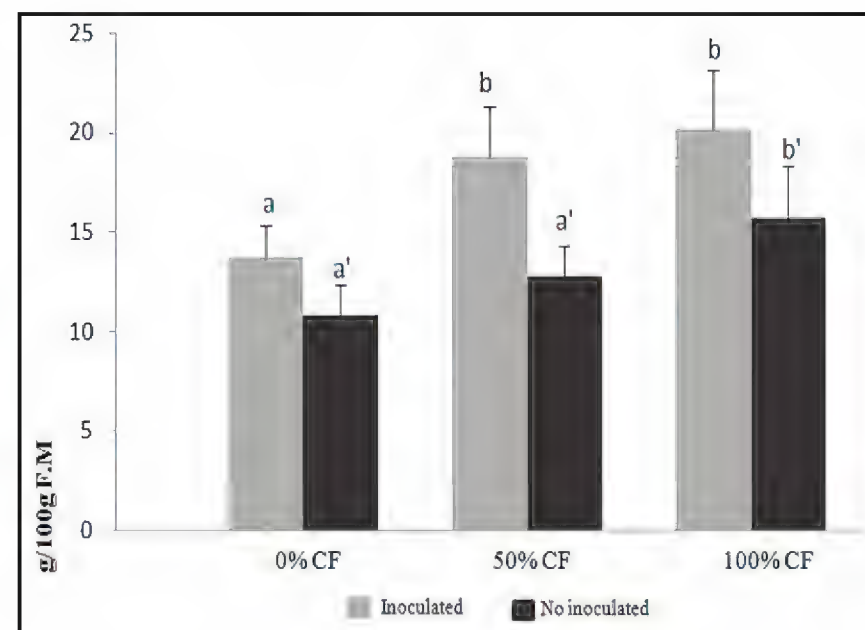


Figure 3. Starch content of potato tubers in the presence of three doses of NPK fertilizer for inoculated and non-inoculated plants.

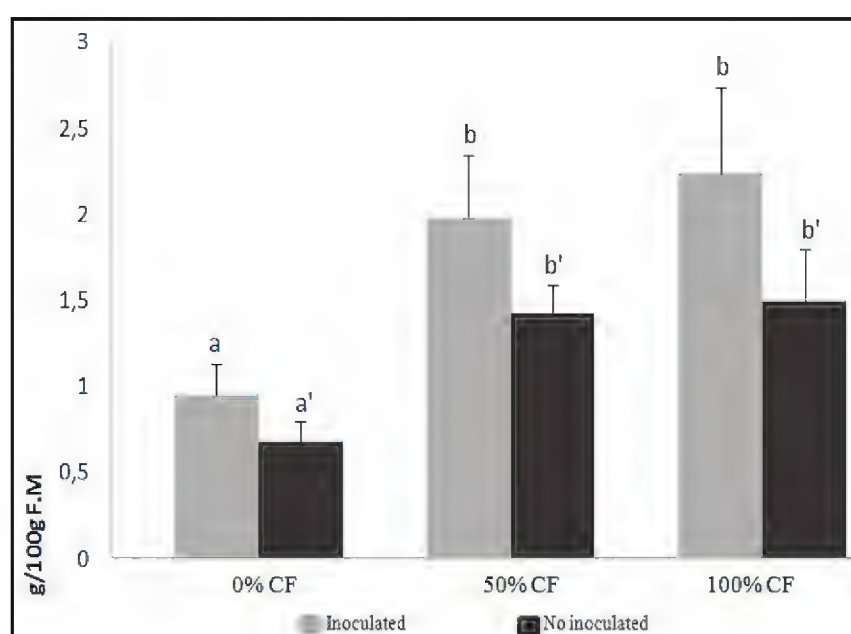


Figure 2. Protein content of potato tubers in the presence of three doses of NPK fertilizer for inoculated and non-inoculated plants.

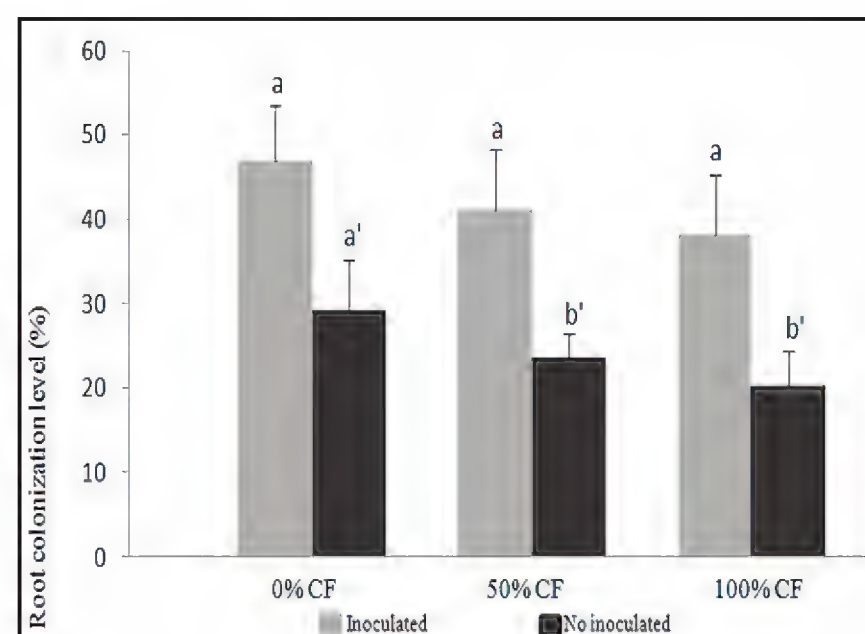


Figure 4. Total, arbuscular and vesicular colonization rates of potato roots in the presence of three doses of NPK fertilizer for inoculated and non-inoculated plants.

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Relationships between macroarthropods assemblages and soil characteristics: data from forest habitats of Central Italy

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ABSTRACT

With this work we wanted to study qualitatively and quantitatively epigeal soil fauna in some forest areas in the Regional Natural Reserve of Monte Rufeno, Lazio (Italy). In addition to the meso- and macroarthropods (quality and quantity) we also examined some chemical parameters of the soil (pH, organic carbon, nitrogen, exchangeable cations, cation exchange capacity, limestone) and physical ones (particle size, apparent density), in order to demonstrate possible relationships between the texture of the soil fauna and the soil itself. The sampled stations consist of mixed oak and were chosen to represent the diversity of these forest formations in the area concerned. The results are structured in a scheme that identifies the soils groups, an analysis of the fauna, and fauna-soil correlations. By the different approaches of statistical analysis conducted to assess the interlinkages between soil characteristics and density of activities of centipedes and of all arthropods, the values of the number of species detected confirm those found in other forest areas of central Italy. As regards the correlations between soil characteristics and density of activity of the Arthropods, it was observed that the latter is positively correlated with the amount of organic substance and, secondarily, with the quantity of clay + silt present in the first layers of the profiles. It was also deepened the study of one taxonomic group (Chilopoda) and, with respect to the results, the number of species that constitutes the taxocenose within the Reserve (19 species), confirmed results found in other forest areas of central Italy. The small skeleton and surface horizon of the soil, do not seem to favor the presence of centipedes.

KEY WORDS

analysis of biodiversity; multivariate analysis; centipedes; macroarthropods soil.

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INTRODUCTION

With a view to safeguarding the Earth's ecosystems, biodiversity and the promotion of sustainable development strategies, soil conservation has certainly a major role since it is home to many forms

of animals, plants, fungi and microorganisms which perform essential functions (production, consumption, mineralization) for terrestrial ecosystems.

In this sense it is meant not only the physical maintenance of the substrate for forest and agricul-

tural productions, but also the maintenance of a high level of “quality”.

In this work we considered arthropods and the centipedes in order to demonstrate possible relationships between the texture/quantitative of the soil fauna and the soil itself.

We decided to study the centipedes as one of the Authors of this work is a scholar of this group of arthropods.

The Chilopoda constitute a class of terrestrial arthropods small in number, represented in the world by about 3300 species (Minelli, 2006), of which 486 in Europe (Stoev & Enghoff, 2011) and 162 in Italy (Foddai et al., 1995; Zapparoli & Minelli, 2005, Zapparoli & Peroni, 2007). It is a taxonomic group of particular biogeographical and ecological interest, since the species are relatively small, generally with narrow geographic area, often with discrete levels of endemic, predators (in particular of small invertebrates), mostly related to the first layers of the soil. They live especially in forest ecosystems, where in many cases are affluent communities, often numerically well represented, of edaphobic, subcorticicolous, and sublapidicolous species, widespread from litorals to over 4000 m above sea level (see also Lewis, 1981; Minelli & Iovane, 1987; Zapparoli, 2006).

Despite the still fragmentary knowledge of taxonomy, geographic distribution and habitat preferences of some species, they may be considered useful ecological and biogeographic indicators.

The Regional Nature Reserve of Mount Rufeno, given its geographical location, within easy to reach mountain ranges rich of preappennines floristic and particular elements (such as as Amiata and Cetona Mountain and eroded forms of the Paglia Valley) suggests a singular interest also in terms of wildlife.

In this area, in fact, coexist northern European mesophilic elements, at the southern limit of their distribution area, next to southern or Mediterranean thermophilic elements, at the northern limit of their range (Papi, 1997; Vigna Taglianti & Cobolli, 1992).

MATERIAL AND METHODS

Study area and sampling sites

Monte Rufeno Regional Nature Reserve covers

approximately 2,892 hectares and falls within the territory of the municipality of Acquapendente, province of Viterbo, Lazio northeastern. The morphology is typically hilly (average altitude 500 m above sea level; Greppe of the Maddalena, 774 m above sea level, Mount Rufeno 734 m, river Paglia 240 m), the substrate consists of turbidite sediments facies Ligure, represented by layers of limestone marl alternating with clays; the stay part consists of flysch sediments of autochthonous whole, as well as small patches of the volcanic Vulsino district, found only in the southern sector of the Reserve (Buonasorte et al., 1988). The whole area is affected by landslides, both ancient and recent; in the landslide, which presents counterslopes, there are small reservoirs that hold water (locally “Trosce”), creating wetlands of natural interest. The surface hydrography is represented mainly by the Paglia River, dividing into two parts the Reserve, which flows into tributaries with torrential regime. The vegetation is strongly shaped by man (Scoppola & Avena, 1992; Blasi, 1994; Scoppola, 1997).

The most relevant vegetational surface by extension and articulation is the oak-dominated *Quercus cerris* L., which occupies approximately 49% of the surface area. This formation, sometimes represented by old coppices, is found mainly in the areas east and west of the Paglia river, from base of the valley to the highest altitudes. This wood assumes different aspects, mesophilic or thermo-Mediterranean, depending on the exposure, the altitude and the substrate. Connected to the facies more mesic of turkey oaks, it is *Castanea sativa* Mill. coppice. Although in the past it was favored by the man, this species is now only in the summit of Monte Rufeno, resulting in formation of a limited extension in which there are also other broadleaf trees *Carpinus betulus* L., *Ostrya carpinifolia* Scop., *Quercus cerris*, *Acer obtusatum* (Waldst & Kit. ex Wild.) Gams., *Fraxinus ornus* L. In connection with the freshest aspects of the Turkey oak is also the mesophilic mixed forest, which develops in suitable slopes, in the valleys or along ditches and streams (Monte Croscione, Troscia of Porcino, Tigna, Fossatello, Tirolle), including *Quercus petraeae* (Mattuschka) Liebl., *Carpinus betulus*, *Ulmus glabra* Huds., *Acer* sp., and *Castanea sativa*. On southern slopes and in the more xeric areas develop irregularly

Quercus pubescens woods, with many open areas where *Spartium junceum* L. and other heat-loving species live frequently. These are secondary formations, kind of replacement of the Turkey oak. In some areas of non-high altitude (Bandita, Africheto) develops a stain in sclerophyllous vegetation, also of secondary origin, presumably replacing a deciduous oak forest, dominated by *Arbutus unedo* L., *Pistacia lentiscus* L., *Viburnum tinus* L., *Smilax asper* L., *Phyllirea latifolia* L. and *Quercus ilex* L.

There is also a extensive reforestation (25% of the surface area), represented by groups of conifers (*Pinus* sp.) planted in the 1950 and 1970 on abandoned agricultural land. On the right bank of the Paglia river are located open formations, represented by abandoned cultivations of little natural interest or grass-pasture attributable to brome grasses. Along the banks of the Paglia there are examples of riparian vegetation, although limited in range, dominated by *Salix* spp., *Alnus glutinosa* (L.) Gaertn., *Populus* spp., *Fraxinus oxycarpa* Bieb ex Willd. and *Quercus robur* L.

The nomenclature of the plant associations to which they refer the woods of the stations follows Scoppola (1997) and Scoppola & Filesi (1997).

Sampling sites. Samplings were found in 11 locations to represent the phytosociological and pedological characteristics of the Monte Rufeno Regional Nature Reserve.

Station 1. Greppe Maddalena, 665 m, exposure WNW, gradient 43%, clay and limestone soil, shallow soil (46 cm), rugged terrain (roots emerging, medium-high erosion: micro ditches), outcropping of rocks virtually absent (1%); high forest transient. *Cerreta xerophile Quercus cerris* and *Quercus pubescens* Willd. The oak woods of this sector of the reserve, although belonging to the floristic structure to *Quercion-pubescent petraeae*, have not been further typed.

Station 2. Field Baglioni, 755 m, exposure NNE, 10% slope, substrate flyschoid sandstone soil with low depth (39 cm), not rugged terrain, rocks outcropping little (2–3%); coppice with reserve trees; oak woods mesophilic attributable to association *Cephalanthero longifoliae-Quercetum cerridis*.

Station 3. Macchione, 595 m, exposure SW, slope 33%, sandstone substrate, soil with low depth

(36 cm), rugged terrain (erosion, micro-ditches), outcropping of rocks absent; coppice with reserve trees. oak woods mesophilic attributable association *Maples obtusati-Quercetum cerridis*.

Station 4. Morto del Loto, 645 m, exposure NE, 10% slope, clay and limestone soil, soil with average depth (> 42 cm), rugged terrain (widespread erosion and channeled), outcropping of rocks virtually absent (1%); coppice with reserve trees, oak woods xerophile attributable to association *Serratulo-Quercetum petraeae* var.

Station 5. Troscia dell'Erba scopina, 560 m, exposure NE, slope 6%, flyschoid-sandstone substrate, soil with low depth (37 cm), not rugged terrain; rocks outcropping virtually absent (1%); coppice with reserve trees, oak woods thermophilic attributable to association *Asparagus tenuifolii-Quercetum cerridis*. A permanent water collection (Troscia) originated from the counter slope of a landslide.

Station 6. Macchia Bruciata, 660 m, exposure W, 2–3% slope, clay and limestone soil, soil with low depth (36 cm), not rugged terrain, rocks outcropping absent; coppice with reserve trees. Oak wood xerophilous, with a prevalence of *Quercus ilex* L. attributable to the association *Orno-Quercetum ilicis* var.

Station 7. Monte Rufeno, 660 m, exposure E, slope 2–45%, flyschoid-sandstone soil, soil with low depth (34 cm), moderately rugged terrain (erosion: widespread) and outcropping of rocks virtually absent (1%); coppice under conversion to high forest. Wood mesophilic with prevalence of *Castanea sativa* with affinity for the association *Digital-Castanetum*.

Station 8. Monte Rufeno, 650 m, exposure NE, slope 44%, flyschoid-sandstone soil, soil with low depth (32 cm), moderately rugged terrain (erosion: widespread) and outcropping of rocks virtually absent (1%); high forest transient. Wood mesophilic including *Castanea sativa* and other hardwoods with affinity for the association *Digital-Castanetum*.

Station 9. Lame, 500 m, exposure E, slope 25–38%, limestone substrate, soil with low depth (25 cm), rugged terrain (presence of stones, strong and widespread erosion) rocks outcropping common (3–15%); coppice with reserve trees. Oak wood thermophilic attributable to association *Asparagus tenuifolii-Quercetum cerridis*.

Station 10. Bandita, 300 m, exposure S, gradient 8–23%, sandstone substrate, soil with low depth (27 cm), moderately rugged terrain, rocks outcropping absent; coppice with reserve trees. Oak wood xerophilous with a prevalence of *Quercus ilex* attributable to association *Orno-Quercetum ilicis* var.

Station 11. Fosso del Molino-Paglia River, 240 m, exposure NW, slope <2%, alluvial soil, soil with low depth (>30 cm), not rugged terrain, rocks outcropping absent; coppice with reserve trees. Hydric riparian forest with a prevalence of *Alnus glutinosa* and *Quercus cerris* with *Salix* and *Populus* spp., attributable to association *Aro italici-Alnetum glutinosae*.

Sampling method for Arthropods assemblages

The material examined was achieved by sampling with pitfall traps (see Wytwer, 1995, 2000; Bainsi et al., 2016).

Samplings (11 stations) with pitfall traps were performed each month during the period February 2001–February 2002. In each station were placed six traps 10–20 m away from each other, according to the morphology of the terrain and the penetrability of the forest; each trap was constituted by a glass of plastic of 9 cm in diameter and of 50 cc capacity, to which a drainage hole has been practiced about 1 cm from the edge; the traps were baited with a solution of vinegar and formalin 4%.

For each species it was calculated the density of activities (DA) in accordance with Mazzei et al. (2015): $DA = [n^\circ \text{ individuals} / (\text{trap} \times \text{days})] \times 10$. For the calculation were used only adults. In order to obtain a picture of the trend of seasonal activity, for each species, the values obtained in the individual stations were summed and plotted on the graph.

Diversity Analysis

For each station the following diversity indices were calculated (Magurran, 1991):

- Diversity Index Shannon-Weaver (H') (Magurran, 1991; Bugio, 1999):

$$H' = -\sum p_i \ln p_i$$

Where:

$p_i = (n_i / N)$ is the frequency or proportion of in-

dividuals of each species on the total, n_i being the number of individuals of a certain species, and N is the total of sampled individuals.

\ln = natural logarithm

- Uniformity Index or equipartition (Evenness, E) (Magurran, 1991; Bugio, 1999):

$$E = H' / \ln S$$

Where:

S = number of species

H' = Shannon-Weaver Index

- Jaccard similarity index (J) (Magurran, 1991; Bugio, 1999):

$$J = j / (a + b - j)$$

Where:

a = number of species at site a

b = number of species at the site b

j = number of species in common.

Soil sampling and analysis

Compilation of country cards. For detection of the soils the following field card has been used, in which the main elements were listed and described (see below). These cards allow to make a detailed and macroscopic description of the station, and the soil.

Topography - altitude, slope and site exposure.

Morphology - provides a short description of the landscape, its basic forms and dynamics.

Lithology - information on the type of pedogenetic substrate (bedrock).

Structure - spatial organization of soil aggregates; With an indication of the “type”, “degree” and “class.” Type: granular, sub-angular polyhedral, multifaceted angular, prismatic, laminated. Grade: weak, moderate, strong. Class: fine, medium, large, very large.

Porosity - percentage of visible voids present in a unit surface area of the layer, mixture of macropores (> 60 μ) and slits (> 0.1 mm).

Rockiness - the presence or absence of rock outcrops on the soil surface. It is estimated as a percentage (not rocky <2%, poor 2–10%, rockiness 10–25%, high 25–50%, highest 50–90%)

Stoniness - Indicates the presence on the soil of stones divided in 3 size classes: small diameter >7.5 cm, average 7.5 to 25 cm and large >25 cm; while the presence of 5 classes are expressed in percent-

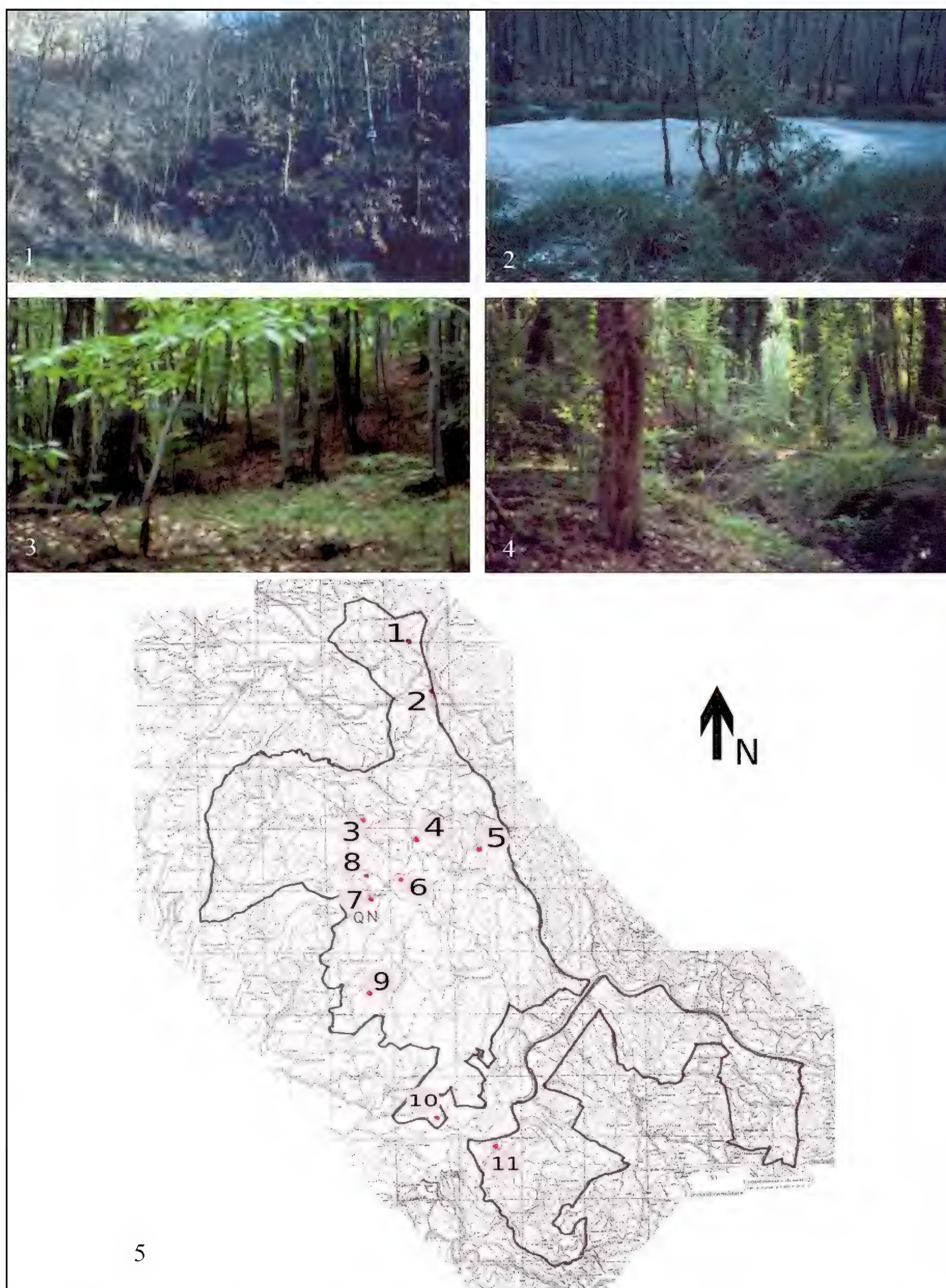


Figure 1. Station 1, Greppe Maddalena. Figure 2: Station 5, Troscia dell'Erba scopina. Figure 3: Station 8, Monte Rufeno; Figure 4: Station 11, Fosso del Molino-Paglia River. Figure 5. Map of the distribution of the sampling sites within the Regional Natural Reserve of Monte Rufeno, Lazio (Italy).

age (absent <0.01%, poor 0.01–0.1%, common 0.1–3%, high 3–15%, highest 15–90%).

Weaving - Represents the percentage between the size classes: sand-silt-clay. Textural classes are established by the triangle of Muller (Soil Taxonomy, USDA).

Color - is determined with tables Munsell Soil Color Chart. The three variables that characterize a given color are given by “Hue”, “Value” and “Chroma”.

Chemical-physical analysis of the soil: analytical procedures. Chemical properties of the soil used primarily for its characterization are as follows using the “Methods of Analysis of Soil Chemistry” National Observatory MIPAF pedological and soil quality (Violante, 2000):

soil reaction (pH): with the potentiometric method and water/land ratio equal to 2:5;

carbonate content: gas-volumetric method by the determination of the CO₂ released using the calcimeter Dietrich-Fruhling;

the cation exchange capacity (CEC): the sum of the cations that in minerals and organic colloids of a soil can be exchanged by means of an ammonium acetate solution.

bulk density: determined through the use of a known volume sampler, thus making the ratio of the weight of the dry ground and the volume of the sampler;

organic carbon (C): the method used is Walkley-Black (Walkley & Black, 1934),

total nitrogen: method Kjeldahl (see Kjeldahl, 1883)

C/N ratio: the ratio between the percentages of organic carbon and nitrogen;

soil texture: particle size determination of mineral soil particles (diameter <2mm) grain size classes according to USDA. Eliminated the organic component, the determination of the clay fraction was derived by densimetric analysis, with the method of Bouyoucos hydrometer (see also Bouyoucos, 1935); the sand was obtained by sieving, while silt by difference.

Skeleton: ‘was obtained by sieving ($\varnothing > 2$ mm) a known amount of land and is expressed as a percentage ratio.

For the classification of soils was used the Soil Map of the Monte Rufeno Nature Reserve (Biondi et al., 2000), it is based on the Soil Taxonomy method that was developed by the Soil Survey Staff

of the Department of Agriculture US ((USDA, 1972, 1975, 1998,1999).

Climatic parameters

In order to characterize this work from a point of view of soil and climate, and in particular define availability of soil water, was used the Thornthwaite method (Thornthwaite & Mather, 1957).

This method allows to do the “soil water budget” and then calculate indices defining the climatic characteristics of the territory. The climate classification according to Thornthwaite method, has as its central point the determination of two indices: “Ih = humidity index”, and “Ia = aridity index” which are derived from the “soil water budget”. This assessment is done by calculating the temperature (T), precipitation (P), the potential (Etp) and real (ETR) evapotranspiration for each month and then year for a given territory.

Thornthwaite poses that the potential monthly evapotranspiration of an area is a function of temperature (T); the average air is calculated to the following exponential relationship: $Etp = 1.6 (10^{T/Ic})^\alpha$, where “Ic” is the annual index heat obtained from the sum of the 12 monthly indices of the heat, and “ α ” is represented from the index annual calorie “Ic”. Taken precipitation “P”, the temperature “T” and the water supply useful soil “Ru” (calculated using the “Salter-Wiliams” formula, Persicani, 1989), it possible to calculate, by Thornthwaite formula, the “soil water budget”. From this budget there are two important parameters in mm of water: the water deficit of the soil “D” and the soil water surplus “S”; with these values the two climate indices (“Ih = humidity index”, and “Ia = aridity index”) were calculated.

By the term evapotranspiration it means the total water that is transferred into the atmosphere by evaporation from the soil and from liquid mirrors, and for transpiration from plants. For the practical purposes little matters to know the quantity of water passing in one way or another since the global phenomenon is the result of overlap of both phenomena. Other things being in equal conditions, with increasing water availability in the soil will increase the value of evapotranspiration, but not indefinitely. There will be an evapotranspiration limit which will not be exceeded even for more

availability of water; this limit is called the potential evapotranspiration Etp.

We define:

ETR-real Evapotranspiration: sometimes also called evapotranspiration current. It is the result of the interaction vegetation-atmosphere- soil that really happens.

1. power evaporating atmosphere;
2. vegetation (type, development and vegetative stage);
3. soil water content

ETP-Potential evapotranspiration: this evapotranspiration depends on the same factors of the "real", however, when the soil water content does not constitute a limiting factor for it. The availability of water in the soil should be equal to or greater than the amount of water that the "soil-vegetation-atmosphere" system is able to evaporate.

Ru-useful reserve: this value (expressed in mm) will normally be achieved by making the difference between the humidity of the soil at field capacity and moisture at the wilting point multiplied by the apparent density of the soil (g/cm^3); in this study we have used the empirical formula of Salter et al., (1966) which takes into account the percentages of sand, silt and organic carbon in the soil, which gave acceptable results.

The following climatic indices were calculated: Ia (aridity Index), Ih (humidity Index), Ig (global humidity Index). Soil water balance values are expressed in the Table 1.

The set of temperature and precipitation data are referred to the thermo-pluviometric stations of Monaldesca (altitude above sea level 700 m) and Acquapendente (altitude above sea level 390 m), considered for a 10-year time interval. The rainfall values were averaged; while for the values of temperature, being the survey sites distributed at different heights, they have been adjusted according to the thermal gradient, and then averaged. Therefore each station study differs from the other.

Data analysis

The two sets of data, wildlife and soil, have been

studied by relating them to each other by means of multivariate statistics (Figure 6).

The choice of the soil variables to be used in the model, can be made in a logical manner, on the basis of what has been observed in the analysis of the main components, by entering only some variables strongly correlated with the first components of the model through the analysis of statistical indices such as r-squared, standard error of the estimate, standardized coefficients etc. Or you can resort to some of the proposed variable selection by the software techniques that provide for the inclusion or exclusion of independent variables, on the basis of statistical criteria that anyway tend to maximize the fit of the model. The most used methods are: Stepwise selection, selection Forward, Backward elimination.

The dependent variable of the model that has been used is the density of activities (DA), while, among independent variables, pedological ones are those that best correlate with the model.

In the multivariate statistical analysis, the matrix of correlation coefficients was observed, which did not reveal strong relationships, and later the study was dealt with in more detail using Factor analysis, used to identify a small number of factors that representing synthetically the relationships between a group of related variables.

The factor extraction was carried out by resorting to PCA (Principal Components Analysis). The study of the relationships between variables that describe a series of cases or objects may be faced with the principal component analysis which consists of a particular linear combinations of the observed variables, extracted for subsequent steps.

The first principal component (PC) is a linear combination that collects itself the highest share of common variance between the variables studied. The second PC is a linear combination that collects the maximum amount of residual variance not controlled by the first PC and is not correlated with this. Subsequent PCs explain portions gradually decreasing residual variance and are not related to each other. The number of the extracted components is equal to the number of variables in the study, but the attempt is to minimize the components, trying to make maximum the total variance explained by these.

RESULTS

In addition to the multivariate statistical analysis described in the “Material and Methods” section, numerous other multivariate correlations have been made to correlate faunistic, pedological and climatic data, all possible combinations have been made but none statistically significant results were yielded, probably because of a limited set of consistent data available in a single year of sampling.

Therefore, only the results with statistical significance will be presented here.

Soils groups

The variables considered in the clustering process are: the pH, the CEC, the bulk density, the sum as a percentage of the granulometric classes of clay and silt, the organic substance, the C/N ratio.

The result of the cluster analysis is shown in figure 6 which represents dendrogram of sampling stations as a function of the soil characteristics, using the grouping techniques of the average link between groups.

The dendrogram shows four groupings: group A consists of five stations (2, 5, 7, 8 and 10); group B by two stations (1 and 9); group C by three stations (4, 6 or 3); group D by one (11).

Table 2 shows the average values of the soil characteristics of the four groups. The characteristics that diversify the station 11 from the others are the low values of the organic substance and the amount of clay and silt, while the pH and the apparent density take on higher values.

Group A is characterized by a low pH value; Group B presents high pH, clay + silt and organic matter; group C is characterized by high values of CEC, clay + silt.

Fauna analysis: Arthropoda

Table 4 shows the overall data of the annual activity density and total taxa sampled for each of the eleven examined taken station.

Stations with larger number of sampled taxa (44) are 10 and 3.

The taxa with the highest density of annual activities (DAa) found in the natural reserve, are as follows:

	Stat. 1	Stat. 2	Stat. 3	Stat. 4	Stat. 5	Stat. 6	Stat. 7	Stat. 8	Stat. 9	Stat. 10	Stat. 11
Ru - useful reserve (mm)	105.0	58.00	105.0	140.0	87.08	87.04	87.06	89.09	78.03	56.00	51.02
Etp - potential evapotranspiration (mm)	669.0	684.0	709.0	702.0	714.0	700.0	700.0	701.0	724.0	758.0	769.0
Etr - real evapotranspiration (mm)	553.0	508.0	558.0	575.0	548.0	538.0	540.0	543.0	545.0	540.0	533.0
D - soil water deficit (mm)	146.0	176.0	161.0	127.0	166.0	191.0	159.0	158.0	178.0	217.0	236.0
S - soil water surplus (mm)	503.0	548.0	498.0	482.0	508.0	518.0	516.0	513.0	511.0	515.0	523.0
Ia - aridity index	22	26	23	18	23	27	23	23	25	29	31
Ih - humidity index	75	80	70	69	71	74	74	73	71	68	68
Ig - global humidity index	53	54	48	51	48	47	51	51	46	39	37

Table 1. Results of the calculation of climate indices.

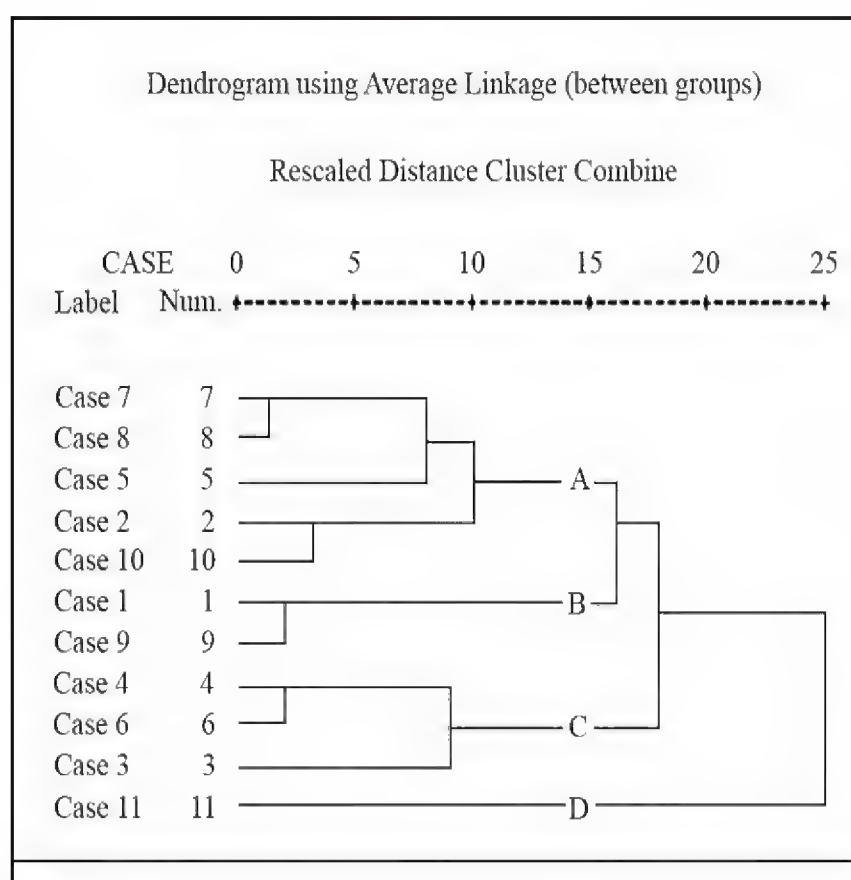


Figure 6. Dendrogram of soil data of sampling stations, using the linkage between groups.

Soil characteristics/ groups of stations	Group A (7-8-5-2-10)	Group B (1-9)	Group C (3-4-6)	Group D (11)
pH	5.98	7.50	6.47	7.80
CEC	25.88	21.90	34.60	28.20
C/N	18.20	22.00	17.00	16.00
Clay+ Silt	52.20	72.50	77.00	40.00
Bulk density	1.20	1.23	1.37	1.52
Organic substance	5.40	6.05	4.37	1.30

Table 2. Soil characteristics (mean values) of the stations groups identified through cluster analysis.

Diptera.....2,289.43
 Coleoptera Staphylinidae.....1,860.58
 Hymenoptera Formicidae.....1,804.28

Other taxa with densities of greater activity of 500.00 are:

Coleoptera Geotrupidae..... 829,07
 Araneae..... 599,03
 Collembola..... 539,78
 Coleoptera Carabidae..... 240,46

The density of monthly activity (DAm) follows the seasonal pattern of temperature. The spring-summer months, from April to September, generally have higher values. It has a peak of DAm values in January–February, most likely due to the sum of the DAm of some stations (Stations 1, 3, 7 and 9, have DAm greater than 100, mainly due to the activities of Diptera).

DAm's minimum values are in October–November, probably due to a sudden drop in temperature during the study period and the beginning of autumn precipitation. In this regard it should be noted that the year 2001 had an extraordinary season because until October, there have been relatively high temperature values (Table 4).

The stations with the density of higher activity are the two chestnut: station 7 Rufeno Muntain (with DAa = 1416.93) and 8 Rufeno Muntain (with DAa = 1350.44). In both, the main contribution is given by the activity of staphylinid beetles, Diptera, ants and beetles Geotrupidae.

The station with lower density of activity is the station 5 Troscia dell'Erba Scopina (with DAa = 678.81).

The density of monthly activity has irregular trend for most of the stations, only some have a Gaussian (normal) trend, although with some “anomalies”:

Station 1, regularity is interrupted by a positive peak in January/February; Station 4, it was found the max in June and the minimum in December/January; Station 8, there was a decrease in density of activity in the months from July to September; Station 11 has a positive peak from August to September. The positive peaks are due:

Station 1 (January/February) to Diptera (DAa = 144.64); Station 4 (in June), Hymenoptera Formicidae (DAa = 103.64); Station 11 (August/September), the rove beetles (DAa = 38.48) and Diptera (DAa = 29.79); Station 3 presents values of DA monthly rather low throughout the year except for February/April period when the greatest contribution of DA comes from Hymenoptera Formicidae (DAa = 144.88) and Diptera (DAa = 58.54). In this station sampling was not carried out from April to May.

stational features	1	2	3	4	5	6	7	8	9	10	11
Vegetation cover	f.C.R.	cm.C.Cn. R	cm.C.R. O	cm.C.Ro. O	cm.C.F. O	cm.C.R. O	f.Ca. m	f.Ca. p.	cm.C. O	cm. L	cm.C.Cb. Fa
pedogen-etic substrate	CM	A	A	Fac	A	Fac	A	A	C	A	al
type of soil	Xop.coll	Xop.d	Xop.t	Xop.coll	Xop.d	Alf.oll	Xop.d	Xop.d	Xop. coll	Xop.t	Fluv.oll
exposure	WNW	NNE	SW	NE	E	W	E	NE	E	S	NE
elevation	665	755	595	645	560	660	660	650	500	300	240
First layer											
pH	7.4	5.6	6.3	6.3	5.6	6.8	6.3	6.1	7.6	6.3	7.8
S.O.	7.5	8.7	14.1	7.7	6.8	4.9	19.3	23.6	16	8.9	2.5
DA	0.77	0.95	0.85	0.78	0.75	1.05	0.74	0.86	0.94	0.77	1.13
clay	28	20.5	34.1	26.8	6.8	16.4	8.8	7.9	26.2	10.8	11.4
CEC	21.2	26.2	35	38.4	28.3	30.4	23.6	30.7	22.6	20.6	23.2
C/N	24	14	14	18	21	19	20	18	20	18	16
First two layers (average weight)											
pH	7.55	5.43	6.3	6.21	5.52	5.86	6	5.87	7.8	6.25	8.04
SO	6.34	3.75	5.91	3.14	3.59	2.61	7.49	7.56	6.65	3.54	1.32
DA	1.22	1,25	1.29	1.48	0.96	1.33	1.15	1.2	1.18	1.24	1.32
clay	31.9	16.64	35.74	23.28	4.6	33.2	8.06	8.12	27.42	5.25	7.8

Table 3. Lithology, soil and vegetation cover of the sampling stations. Abbreviations: Lithology: A: sandstone; to: the recent floods; C: limestones; CM: marl limestone; Fac: flysch clay and limestone. Soils: Alf. oll.: haploxeralf mollic; Fluv. oll.: udifluent mollic; Xop. coll.: xerochrept calcixerollico; Xop. d: xerochrepts district; Xop. t: typical xerochrept. Vegetation cover: C: *Quercus cerris*; Cam: chestnut mixed; Cap: pure chestnut; Cb: *Carpinus betulus*; cm: coppice with reserve trees; Cn: *Ostrya carpinifolia*; f: high forest; F: *Fraxinus oxyphylla*; Fa: *Quercus robur*; L: *Quercus ilex*; O: *Fraxinus ornus*; A: *Quercus pubescens*; Ro: *Quercus petraea*.

Stations Taxa	1	2	3	4	5	6	7	8	9	10	11	Σ
Arachnida Scorpiones	0.52	0	2.46	0	0.36	0	0	0	0	1.19	0	4.52
Arachnida Pseudoscorpionida	1.71	1.49	2.51	2.51	1.21	3.38	3.47	4.65	2.21	7.37	2.88	32.77
Arachnida Opiliones	1.46	5.56	1.33	7.58	2.24	4.16	2.51	4.87	4.76	5.1	4.12	43.69
Arachnida Araneae	56.93	63.54	52.58	55.76	48.3	70.56	46.13	40.57	68.94	46.76	48.95	599.03
Arachnida Acari	6.32	6.75	6.94	1.88	3.46	7.11	6.9	1.02	24.99	8.71	4.65	78.73
Crustacea Isopoda	15.52	2.86	24.38	6.41	6.14	7.68	7.46	15.37	13.73	9.53	28.2	137.27
Crustacea Amphipoda	0	113.79	0.18	15.73	11.83	0	0	0	0	0.12	0	141.65
Chilopoda	6.22	2.78	6.3	5.16	5.48	4.29	5.09	3.65	4.62	8.9	6.19	58.68
Diplopoda	23.14	2.76	0.73	2.69	3.07	3.35	1.27	1.81	1	4.86	2.71	47.37
Collembola	51.12	33.14	37.58	50.33	37.2	45.28	36.73	55.02	40.28	55.47	97.62	539.78
Protura	0	0.67	0	0	0	0	0	0.17	0	0	0	0.83
Diplura	0.72	0.13	0	0	0	0	0	0	0	0.52	0.51	1.89
Exapoda (larvae different orders, pupae)	62.67	33.05	29.81	26.61	32.09	64.46	20.47	30.36	31.1	25.11	32.29	388.03
Zygentoma	5.75	0.16	6.35	4.49	1.48	4.2	3.22	3.41	6.4	11.83	8.39	55.68
Blattaria	3.6	3.71	1.67	8.36	3.27	4.2	4.26	26.29	1.37	0.43	1.74	58.9
Mantodea	0	0	0	0	0	0	0	0	0	0.24	0	0.24
Isoptera	0	0	0	0	0	0	0	0	0	0.17	0	0.17
Orthoptera Celifera	0.52	0	1.17	1.13	0.71	1.61	0.49	0	0.65	0.24	0	6.52
Orthoptera Ensifera Grillidae	34.15	2.45	73.83	32.24	18.84	32	57.93	15.57	0	24.56	0.74	292.31
Orthoptera Ensifera indet.	0.69	0.42	0	0.2	0	0	0.56	5.83	0.17	0	0.15	8.02
Orthoptera indet. (juvenile stages)	0	2.5	0	0.45	0	0	0	0	0	0	0.21	3.15
Phasmatodea	0	0	0	0	0	0	0	0	0	0.24	0	0.24
Dermaptera	0	0	0.13	0	0	0.6	0	0	0	0	0	0.73
Psocoptera	0	0	0	0	0.18	0	0	0	0	0	0	0.18
Heteroptera	0.52	0.99	1.42	0.34	0.63	0.84	0.15	0.13	0.98	0.82	0.86	7.69
Homoptera	1.84	0	3.16	1.17	0.44	1.82	0.41	0	1.4	2.34	0.6	13.18
Hemiptera (juvenile stages)	0.17	0.89	0.31	0.3	0	0.68	0.48	0.27	0.64	1.25	0.2	5.2
Coleoptera (larvae)	4.09	1.02	2.49	1.61	2.71	4.21	4.18	1.45	9.12	2.41	1.31	34.6
Coleoptera Carabidae	12.05	6.51	3.57	19.34	8.57	28.15	46.77	18.11	3.92	50.86	42.6	240.46
Coleoptera Carabidae (larvae)	0.19	0.19	0	0	0.54	0	0	0.37	0.47	0.18	0.36	2.3
Coleoptera Silphidae	0	0	0.17	0	0	0	0.16	0	0	2.41	0	2.74
Coleoptera Silphidae (larvae)	0	0.17	0	0	0	1.94	0	0	0.39	0	0.17	2.66
Coleoptera Staphylinidae	120.29	217.38	113.47	87.06	58.83	97.08	316.26	365.99	152.85	178.83	152.52	1860.58

Table 4/1. Density and total annual activity of taxa and lithology, soil and vegetation cover of the sampling stations.

Stations Taxa	1	2	3	4	5	6	7	8	9	10	11	Σ
Coleoptera Scarabeoidea	0.51	1.5	0	2.49	3.76	1.36	0.84	0.58	5.96	1.23	2.33	20.57
Coleoptera Lucanidae	0.17	0.76	0.5	0	0	0.33	0.17	0.16	0	0	0	2.09
Coleoptera Geotrupidae	32.48	293.08	3.52	8.02	12.04	2.66	149.93	255.68	56.46	15.19	0	829.07
Coleoptera Cetoniidae	0	0.17	0.31	0	0	0.99	0.16	0.16	1.4	0	0.36	3.56
Coleoptera Elateridae	0	0.47	0.24	0	0.72	0	0	0	0.43	0	0.36	2.23
Coleoptera Buprestidae	0	0	0	0	0	0	0	0	0	0	0	0
Coleoptera Lampyridae	1.06	0.15	0.5	0.72	1.27	0.88	0	0.13	0.78	1.47	0.59	7.56
Coleoptera Lampyridae (larvae)	1.23	0	0.37	1.38	0.36	0	0	0.71	0.74	1.29	1.17	7.24
Coleoptera Cantharidae	0	0	0.12	0	0	0	0	0	0	0	0	0.12
Coleoptera Bostrichidae	0	0	0	0	0	0	0	0	0	0	0.2	0.2
Coleoptera Coccinellidae	0	0	0	0.17	0.27	0	0.24	0	0	0	0	0.68
Coleoptera Meloidae	0	0	0	0	0	0	0	0.17	0	0	0	0.17
Coleoptera Tenebrionidae	1.9	8.58	3.19	5.4	5.35	6.74	8.2	7.18	2.96	5.4	3.51	58.41
Coleoptera Cerambycidae	0.5	0.17	0.33	0.26	0.55	0.16	0.47	0.53	0	0.13	0.38	3.49
Coleoptera Chrysomelidae	0	0	0.31	0.17	0	0.21	0	0.29	0	0.34	0	1.34
Coleoptera Brachyceridae	0	0	0	0.13	0	0	0	0	0	0	0.2	0.32
Coleoptera Curculionidae	3.02	1.71	0.17	0.47	3.75	0.25	1.21	5.29	1.18	1.35	2.35	20.74
Coleoptera Scolytidae	0.17	1.73	1.65	0.4	2.63	5.52	0.86	0	0	0.61	0.16	13.74
Coleoptera (different family)	8.58	17.05	9.93	6.12	9.63	6.35	51.61	25.16	11.74	15.42	17.39	178.98
Neuropteroidea	0	0.34	0	0.27	0	0	0	0	0	0	0	0.62
Mecoptera	0	0	0	0	0	0	0	0	0.19	0	0	0.19
Siphonaptera	0.17	0	0	0	0	0	0	0	0	0.17	0	0.34
Diptera	332.26	113.88	234.3	63.27	96.03	186.67	244.96	272.84	387.71	137.29	220.53	2289.73
Trichoptera	0	0	0	0	0	0	0.17	0	0	0	0	0.17
Lepidoptera	38.82	102.1	54.65	53.68	40.34	109.07	61.13	100.34	31.66	19.25	16.8	627.85
Lepidoptera (larvae and chrysalises)	1.22	5.52	2.6	3.91	4.95	10.48	0.56	1.07	1.76	3.89	1.19	37.14
Lepidoptera Lymantriidae (larvae)	0.4	17	0.83	4.8	4.52	3.87	0.32	0.32	0.58	0.31	0	19.73
Hymenoptera Symphyta	0	0.75	0	0	0	0	0	0	0	0	0	0.75
Hymenoptera Apocrita	4.1	4.72	2.77	1.74	4.2	2.63	5.47	1.57	3.79	2.82	4.37	38.17
Hymenoptera Apocrita Formicidae	64.08	70.43	248.85	240.2	238.24	148.95	259.09	148.01	103.46	84.98	198	1804.2 0
Hymenoptera Apocrita Vespidae	0.17	0.17	0.17	0	0.171	0.33	0.16	1.65	0.5	0.5	4.94	10.29
Hymenoptera (different family)	0	0	0.52	0.34	0.89	0.33	0	0.17	0.65	0	3.54	6.44
Density Activity (DA)	900.61	1130.34	938.39	724.71	678.81	875.41	1350.44	1416.93	981.93	742.13	916.32	
N. taxa sampled	40	43	44	41	40	39	38	39	39	44	41	

Table 4/2. Density and total annual activity of taxa and lithology, soil and vegetation cover of the sampling stations.

One might note that this station is very sensitive to the summer drought since the climate anomaly recorded in the year 2001. It should also be pointed out that this station is located in landslide areas.

Related soil fauna: Arthropods. The matrix of correlation coefficients between soil variables and those of wildlife shows little sharing of their variability, with the exception of the variable annual activity density (DAa) which seems to establish a good correlation with the organic matter (Table 5).

Multiple Regression Model. The choice of the independent variables of the model was made on the basis of some statistical criteria offered by the software (Stepwise selection, selection Forward, Backward elimination). All of them have led to the solution which involves the insertion of organic matter variable and variable clay+silt, thus responding to the logical criterion of choice of the variables among those strongly correlated with the first extracted components. In Table 6 is shown an example of the choice of the variables according to the Backward elimination method that allows to follow, step by step, the elimination of the less representative variables, pending the resolution of the best model possible.

The model chosen is then the number 5: $DA = 1126.32 + 8.636 (\text{clay+silt}) + 76.86 \text{ organic matter}$.

Discussion: Arthropods. Given the limited number of experimental data used, the model can not be used for predictive purposes but only for a further confirmation that the soil variables that have had a certain influence on the activity densities variable are the organic substance and the content of clay + lime of the observed sites.

Similar studies conducted in two stations of Latium, Circeo National Park (LT) area of Canino (VT), respectively in Mediterranean plant formations and olive groves in an agro-ecosystem (Calvario et al., 1986; Zapparoli & Jona Lasinio, 1993) have shown that beetles, springtails Formicidae and arachnids are the most numerous groups.

In the agro-ecosystem with olive trees there are more numerous beetles, carabids and staphylinids (DAa carabids = 40, DAa staphylinids = 26, DAa

ants = 65, other groups less than 30, instead DAa values of the present work are: carabids = 240, staphylinids = 1860, ants = 1804.28) (Zapparoli & Jona Lasinio, 1993). The beetles and araneids have an almost uniform presence throughout the year, but the latter are more abundant in spring. The Collembola are more present in winter–spring. As for centipedes, in the Circeo Park, the highest number of species are collected in plain woods while the poorest stations consist of high and low bush (Minelli & Zapparoli, 1986).

Analysis of fauna: Centipedes

The species of centipedes collected in the Regional Natural Park Rufeno Mountain are 19, distributed (Table 7) in four orders and six families. It is a fauna consisting largely of species with European distribution with some endemics of the Apennines (*Lithobius cassinensis* and *Eupolybothrus fasciatus*).

As a whole, the centipedes community in the reserve has a number of species comparable to that of other forest ecosystems of central Italy; 17–20 species were sampled in Lepine Mountains (southern Lazio) (Zapparoli 2007; Zapparoli & Peroni, 2007); 21 species were collected in the area of Ausoni and Aurunci, Lazio (Zapparoli, 1994) 18 species are known in the forest of the Circeo National Park, Lazio (Minelli & Zapparoli, 1994).

The station with the highest number of species is 11, with eight species, follow the Station 8 and Station 9, both with seven species. The number of species sampled in the other stations oscillates from a minimum of four to a maximum of six (Table 8).

The species common to all stations is *Eupolybothrus fasciatus*, well represented with numerous individuals, especially to forest ecology in the Apennine distribution (Zapparoli, 1992, 1994)

The following stations are characterized by the exclusive presence of:

Station 4: *Stigmatogaster gracilis*

Station 5: *Geophilus carpophagus*

Station 7: *Lithobius tricuspis*

Station 8: *Cryptops anomalans*

Station 8: *Cryptops hortensis*

Station 9: *Himantarium gabrielis*

Station 11: *Strigamia crassipes*

		Sum	pH	CEC	C/N	clay+silt	bulk density	Organic matter (SO)
Sum	Corr. di Pearson							
	Sig. (2-code)							
	N							
pH	Corr. di Pearson	-.100						
	Sig. (2-code)	.770						
	N	11						
CEC	Corr. di Pearson	-.128	-.275					
	Sig. (2-code)	.707	.414					
	N	11	11					
C/N	Corr. di Pearson	-.139	.284	-.457				
	Sig. (2-code)	.683	.398	.157				
	N	11	11	11				
clay+silt	Corr. di Pearson	-.328	.130	.356	.154			
	Sig. (2-code)	.324	.702	.282	.652			
	N	11	11	11	11			
bulk density	Corr. di Pearson	-.259	.422	.350	-.401	-.098		
	Sig. (2-code)	.443	.196	.291	.222	.774		
	N	11	11	11	11	11		
Organic matter (SO)	Corr. di Pearson	.658*	-.117	-.066	.154	.245	-.539	
	Sig. (2-code)	.028	.733	.847	.651	.467	.087	
	N	11	11	11	11	11	11	

Table 5. Relationship. Sum = density of activities; * = The correlation is significant at the 0.05 level (2-tailed).

Model	R	R-square	R-square correct	standard error of estimate
1	.862 ^a	.742	.355	194.1540
2	.860 ^b	.740	.481	174.2783
3	.859 ^c	.737	.562	160.1036
4	.850 ^d	.723	.604	152.2338
5	.830 ^e	.689	.611	150.8268

Table 6. Choice of variables according to the method of Backward elimination; a = estimators: (Constant), organic matter (SO), CEC, pH, C / N, clay+silt, bulk density; b = estimators: (Constant), organic matter, CEC, pH, C/N, clay + silt; c = estimators: (Constant), organic matter, pH, C / N, clay + silt; d = estimators: (Constant), organic matter, C / N, clay + silt; e = estimators: (Constant), organic matter, clay + silt.

		Not standardized coefficients		standardized coefficients		
		B	Standard error	Beta	t	Sig.
1	(Constant)	1114.023	1063.377	-	1.048	0.354
	pH	62.916	131.547	0.197	0.478	0.657
	CEC	4.840	17.176	0.114	0.282	0.792
	C/N	-17.439	5.470	-0.215	-0.628	0.564
	clay+ silt	-9.444	5.470	-0.570	-1.727	0.159
	Bulk density	-139.851	825.657	-0.083	-0.169	0.874
	Organic matter (SO)	79.886	31.424	0.817	2.542	0.064
2	(Constant)	1016.979	804.129	-	1.265	0.262
	pH	46.721	81.099	0.146	0.576	0.590
	CEC	3.305	13.096	0.078	0.252	0.811
	C/N	-15.480	22.678	-0.190	-0.683	0.525
	clay+ silt	-9.165	4.681	-0.553	-1.958	0.108
	Organic matter (SO)	82.679	24.010	0.846	3.443	0.018
3	(Constant)	1164.083	508.862	-	2.228	.062
	pH	41.039	71.574	0.128	0.573	0.587
	C/N	-18.290	18.149	-0.225	-1.008	0.352
	clay+ silt	-8.535	3.638	-0.515	-2.346	0.057
	Organic matter (SO)	81.584	21.694	0.834	3.761	0.009
4	(Constant)	1371.418	340.428	-	4.029	0.005
	C/N	-15.233	16.495	-.187	-0.923	0.386
	clay+ silt	-8.252	3.427	-0.498	-2.408	0.047
	Organic matter (SO)	79.147	20.228	0.809	3.913	0.006
5	(Constant)	1126.323	211.222	-	5.332	0.001
	clay+ silt	-8.636	3.370	-0.521	-2.562	0.034
	Organic matter (SO)	76.876	19.893	0.786	3.865	0.005

Table 7. Correlations. Sum=density of activity; * = the correlation is significant at the 0.05 level (2-tailed).

species/station	1	2	3	4	5	6	7	8	9	10	11
<i>Eupolybothrus fasciatus</i> (Newport, 1845)	11	4	16	11	11	10	4	4	8	8	5
<i>Eupolybothrus grossipes</i> (C.L. Koch, 1847)											4
<i>Eupolybothrus nudicornis</i> (Gervais, 1837)	2		1								
<i>Lithobius cassinensis</i> (Verhoeff, 1925)	2	1	3	1	6	3		1	1	7	5
<i>Lithobius castaneus</i> (Newport, 1844)	2	2					1		3		2
<i>Lithobius forficatus</i> (Linnaeus, 1758)							2	6			1
<i>Lithobius lapidicola</i> (Meinert, 1872)									1	1	1
<i>Lithobius tricuspis</i> (Meinert, 1872)							1				
<i>Lithobius tylopus</i> (Latzel, 1882)	4						7	1			
<i>Cryptops anomalans</i> (Newport, 1844)								1			
<i>Cryptops hortensis</i> (Donovan, 1810)								1			
<i>Cryptops parisi</i> (Brölemann, 1920)				2	2	1	1	1	1	1	1
<i>Himantarium gabrielis</i> (Linnaeus, 1767)									2		
<i>Stigmatogaster gracilis</i> (Meinert, 1870)				1							
<i>Schendyla nemorensis</i> (C.L. Koch, 1837)		2				2					
<i>Clinopodes flavidus</i> (Koch, 1847)					1					1	
<i>Geophilus carpophagus</i> (Leach, 1814)					1						
<i>Geophilus richardi</i> (Brolemann, 1904)	3				2	1			1		
<i>Strigamia crassipes</i> (C.L. Koch, 1835)			1			1					1
tot. n° species	6	4	4	4	6	6	6	7	7	5	8
tot. n° specimens	24	9	20	15	23	18	16	15	17	18	20

Table 8. Natural Reserve of Rufeno Mountain: list of species of centipedes in the stations examined and number of individuals collected.

Stations	1	2	3	4	5	6	7	8	9	10	11
H'	1.53	1.27	0.76	0.82	1.40	1.34	1.48	1.61	1.58	1.20	1.97
E	0.9	0.9	0.5	0.6	0.8	0.8	0.8	0.8	0.8	0.7	0.9

Table 9. Value of Shannon & Weaver index (H') and Evenness (E) in centipedes from the examined stations

These are generally forest elements, more or less closely linked to *Quercus* formations in central and central-Northern Italy, except for *Himantarium gabrielis* species of avventism (Zapparoli, 1992).

Diversity index of Shannon & Weaver (H') and Evennes (E). In Table 9 are reported the values of the Index of diversity of Shannon & Weaver (H') and Evennes (E) of the chilopods taxocene of the various sampled stations. The index of Shannon & Weaver (H') has the highest value in the station 11. Fosso del Molino-Paglia ($H'=1.97$), while the lowest value is observed in Station 3. Macchione ($H'=0.76$) and Station 4. Morto del Loto ($H'=0.82$). This result is probably linked to the fact that both stations are rather degraded with landslide layers. Only four stations have values of H' greater than 1.53.

The parameter (H') would not seem linked to an altitudinal gradient, mesophilic/xerophilous or with soil characteristics detected for each station.

The Evennes follows the trend of H' , except for Station 2. The highest value is in station 11 (0.95).

Jaccard similarity index. Table 10 shows the index values of similarity of Jaccard calculated for eleven sampling stations. From this analysis it can be deduced that the most similar stations are 5 and 10, 9 and 11, 7 and 8, 1 and 2.

Through cluster analysis it was constructed a dendrogram (Fig. 7) in which the hierarchical classification of the stations was carried out using the average bond method (UPGMA). Through this analysis it would appear possible to highlight three groups of stations:

- 1) stations 7 and 8
- 2) stations 11, 9, 6, 10, 5, 4
- 3) stations 1, 2, 3

The groups of stations detected by the dendrograms, are only moderately correlated with some soil characteristics of the same stations.

Related Fauna soil: Centipede. Comparison of faunal and soil data shows an inversely proportional correlation between the number of individuals in each station and the presence of skeleton in the first soil layer.

It has been found that in the stations 1, 3 and 11, where the presence of individuals is greater than 20 (all other stations have fewer than 20 in-

dividuals), the skeleton is small and low, the only exception being station 5 which has 23 individuals with a small and common skeleton (Table 11).

Analysis R^2 . All weak (Fig. 8).

Increasing the value of the granulometric band is there a direct proportionality, of course. This data is confirmed by inverse proportionality with clay and confirmed by the relationship of sandy + silt.

The organic substance, bulk density and pH, have no influence.

So centipedes prefer a generally porous soil.

The humidity index (I_h , calculated with Thornthwaite formula) is inversely proportional to the density of activity. This is confirmed, albeit weakly, by both the evapotranspiration (ETR), which is directly proportional, and the soil water deficit that it is also directly proportional.

In conclusion it appears that the DA of chilopods increases in porous soils and where there is stagnant humidity.

Discussion: Centipedes. Based on the statistical analysis of most representative soil data (pH, CEC, bulk density, organic matter, granulometric sand clay and silt, C / N), the eleven sampling stations are subdivided into four groups.

In the first group are represented the stations that have a sandstone lithological substrate or Flisch arenaceous characterized by a pH subacid (stations 2, 5, 8, 7, 10).

In the second group are placeable stations with soils formed on Flisch clay substrate, characterized by an increased CEC of 30 meq (stations 1, 9).

The third group consists of the stations with soils in which carbonates are present, in fact, the pH is greater than 7.4 (stations 3, 4, 6).

The fourth is replaced by a single station placed on the recent floods (station 11. Fosso del Molino-Paglia).

An alternative to this is to write a general description of the soils in the Reserve.

From the various statistical analysis approaches to verify any correlation between soil characteristics and the density of activity of the centipedes, the number of species that make up the taxocenose of the Reserve is 19, a value confirming those found in other forest areas in Central Italy.

The station with greater number of species is the

stations	1	2	3	4	5	6	7	8	9	10	11
1	1	█		█	█	█	█	█	█	█	█
2	0.43	1	█	█	█	█	█	█	█	█	█
3	0.43	0.33	1	█	█	█	█	█	█	█	█
4	0.25	0.33	0.33	1	█	█	█	█	█	█	█
5	0.33	0.25	0.25	0.42	1	█	█	█	█	█	█
6	0.33	0.42	0.42	0.42	0.5	1	█	█	█	█	█
7	0.33	0.25	0.11	0.25	0.2	0.2	1	█	█	█	█
8	0.3	0.22	0.22	0.37	0.3	0.27	0.44	1	█	█	█
9	0.44	0.37	0.22	0.37	0.44	0.44	0.3	0.27	1	█	█
10	0.22	0.16	0.28	0.5	0.57	0.37	0.22	0.33	0.5	1	
11	0.25	0.33	0.33	0.33	0.27	0.4	0.4	0.36	0.5	0.44	1

Table 10. Jaccard Index values in the comparison between the examined stations

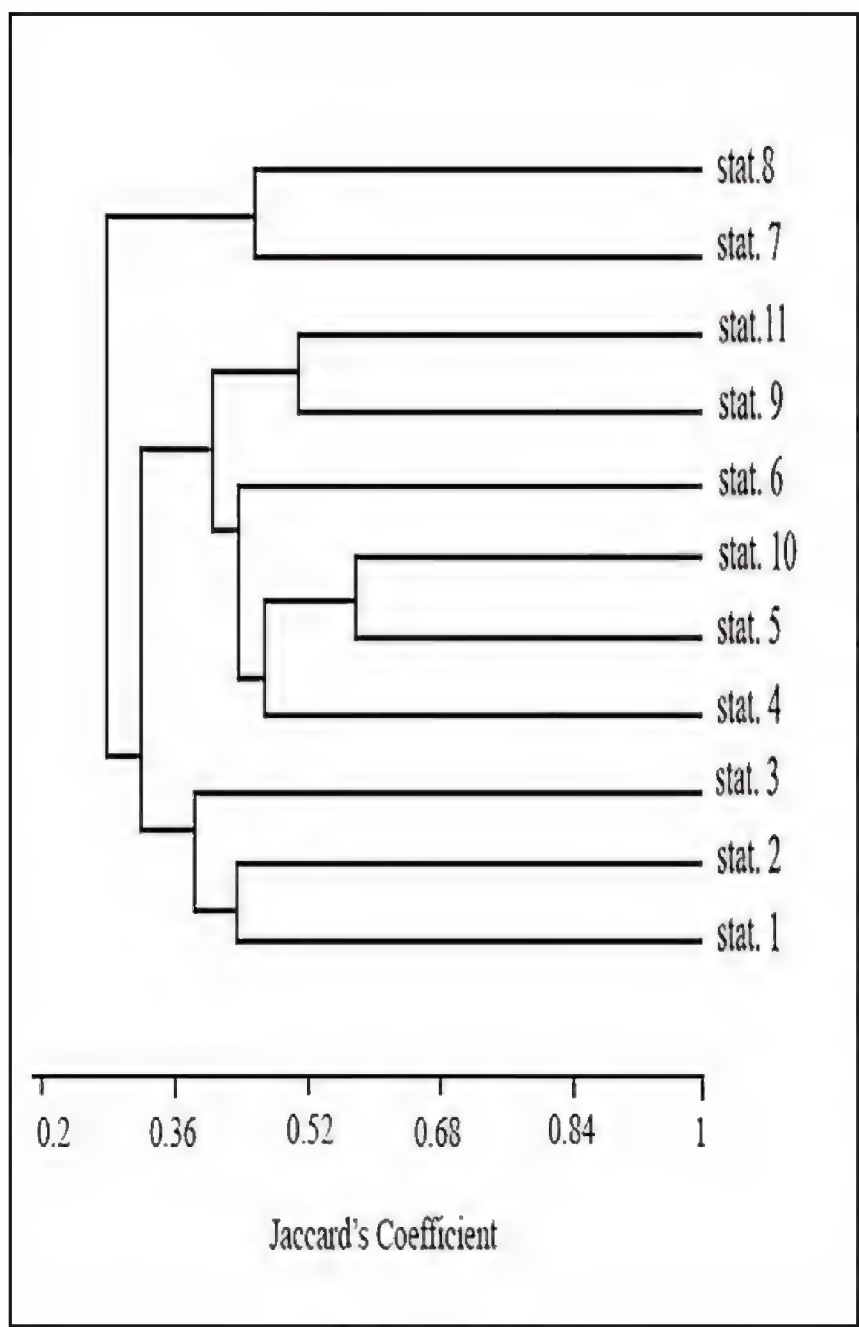


Figure 7. Dendrogram similarity between taxocenoses of centipedes in the Natural Reserve of Rufeno Mountain.

11, confluence Fosso del Molino-River Paglia (8 species) which is located on an alluvial soil with a high sand content.

The common species at all stations is *Eupolybothrus fasciatus*, species of forest ecology, Apennine endemic, while some species, however showing European chorology, have been found exclusively in some stations.

In conclusion, it appears that the DA chilopods increases in porous soils and where there is no stagnation of humidity, and that the small skeleton, and the horizon of the soil, do not seem to favor their presence.

Regarding the diversity of species of centipedes, this was analyzed by the index of Shannon-Weaver, which shows that it is higher in station 11.

While the lowest values of diversity index were found in stations 3. Macchione and 4. Morto del Loto, which are placed in areas affected by land-

station	n° species	n° individual	skeleton
1	6	24	meager, small
2	4	9	none; then plenty, medium/small
3	4	20	none; then meager, small
4	4	15	none; then common, small
5	6	23	none; then common, small
6	6	18	none; common, small
7	6	16	none; common, small
8	7	15	none; common, small
9	7	17	none; plenty, medium
10	5	18	none, frequent, small; frequent, small and medium
11	8	20	none; meager, small, very abundant; gravel

Table 11. Natural Reserve of Rufeno Mountain: centipedes and soil skeleton (first 30 cm, layers A + B). Legend: none <1%; meager 1–5%; Common 5–15%; frequent 15–35%; plenty of 35-70%; very abundant > 70%.

slides consequence of a considerably degraded vegetation.

CONCLUSIONS

To conclude, this study, although conducted for a whole year and for a large number of stations, is still preliminary and partial in nature, as has been investigated only a particular portion of the area of the fauna attached to a precise soil layer, and has maintained an identification taxonomic level not lower than that of the family.

However, the huge amount of collected invertebrates, 58,651 specimens, referring to no less than 3 phyla (Arthropoda, Annelida, Mollusca), 5 classes, 34 orders, more than 23 families of beetles and centipedes for 6 families of centipedes, constitute a important potentially usable

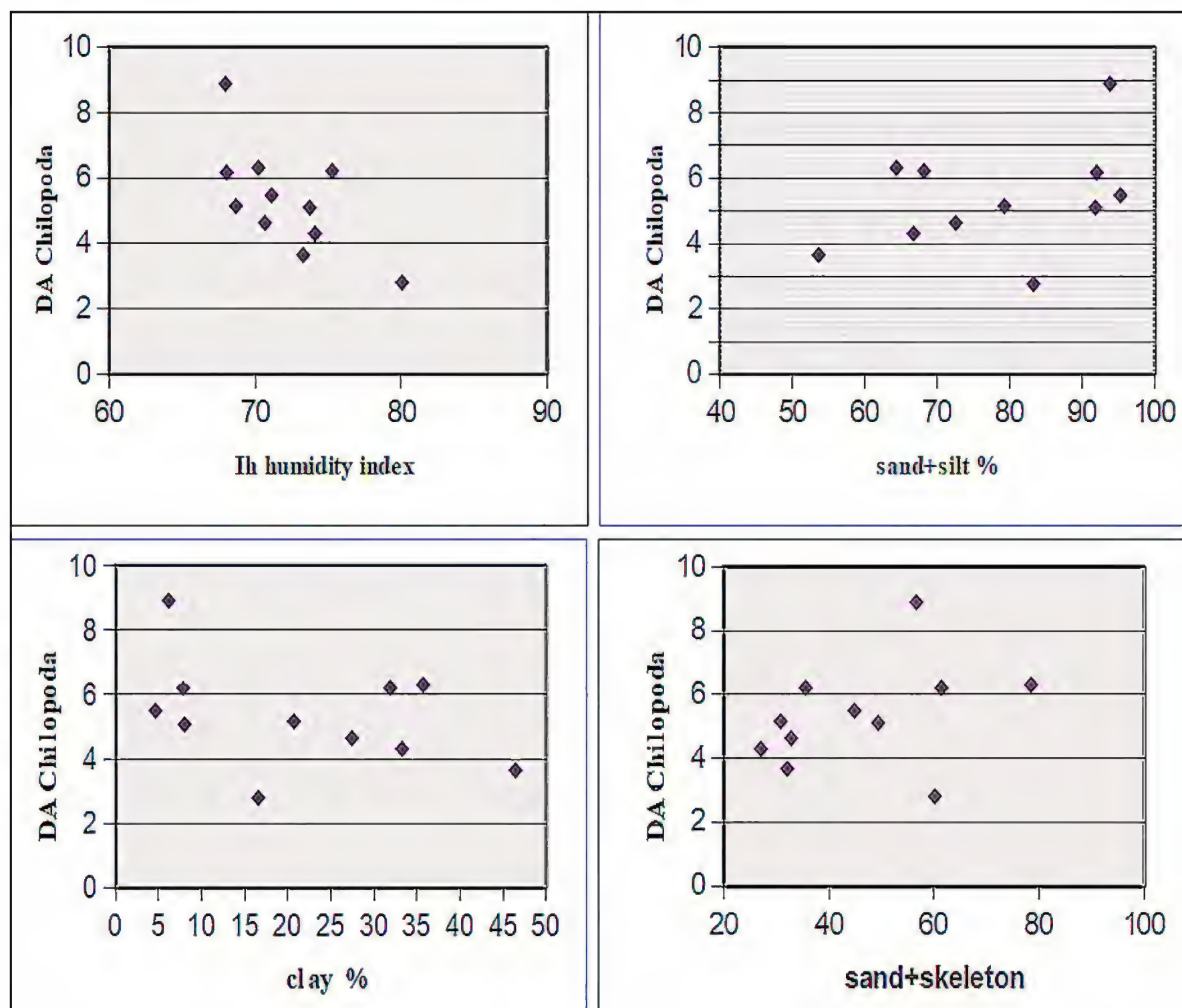


Figure 8. Graphs that relate the activity density of Chilopoda and physical soil parameters: skeleton+sand, clay, sand + silt, and a climate parameter moisture.

material for the continuation of research in the protected area.

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A new genus record of *Yasuhikotakia* Nalbant, 2002 (Teleostei Botiidae) and a new species record of *Brachydanio* Weber et de Beaufort, 1916 (Teleostei Cyprinidae) to China

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ABSTRACT

Two species of small freshwater fishes of Botiidae and Cyprinidae families of SE Asia were collected during field ichthyofauna surveys in lower Lancangjiang, Mekong basin before raining season in 2015. Both *Yasuhikotakia lecontei* (Fowler, 1937) (Teleostei Botiidae) and *Brachydanio rosea* (Fang et Kottelat, 2000) (Teleostei Cyprinidae) are new records to China, and the genus *Yasuhikotakia* Nalbant, 2002 is firstly recorded in China. The occurrence of the two species in Xishuangbanna Prefecture, Yunnan Province is most likely a natural extension of their distributional range. The meristics and measurements of the specimens, diagnosis, photographs, radiographs and distribution map of the two newly recorded species are provided.

KEY WORDS

Yasuhikotakia lecontei; *Brachydanio rosea*; New record; Mekong basin; Southwest China.

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INTRODUCTION

The Southeast Asian fish genus *Yasuhikotakia* Nalbant (2002) is a group of beautiful ornamental fishes of family Botiidae (Teleostei Cypriniformes). There are totally seven valid species in Mekong, Chao Phraya and Maeklong basins in Thailand, Laos and Cambodia (Kottelat, 2012; 2013), viz. *Yasuhikotakia caudipunctata* (Taki et Doi, 1995), *Y. eos* (Taki, 1972), *Y. lecontei* (Fowler, 1937), *Y. longidorsalis* (Taki et Doi, 1995), *Y. modesta* (Bleeker, 1864), *Y. morleti* (Tirant, 1885) and *Y. splendida* (Roberts, 1995), respectively.

Danio Hamilton (1822) (Teleostei Cyprinidae) is a diverse group of small ornamental fishes throughout SE Asia including Yunnan of China and Indian

Subcontinent. Fang (2003) and Fang et al. (2009)'s phylogenetic works based on morphological and molecular data show that *Chela* F. Hamilton, 1822 + *Laubuca* Bleeker, 1859, *Esomus* Swainson, 1839, *Danio*, and *Devario* Heckel, 1843, with the addition of *Microdevario* Fang, Norén, Liao, Källersjö et Kullander, 2009 are a monophyletic group, and that *Danio* and *Devario*, are not sister groups. 'Danio' was further divided into two major genera by Fang (2003), viz. *Danio* and a new erected genus *Devario*. Based on Fang et al. (2009) and other published molecular trees, Kottelat (2013) preferred to further divide 'Danio' into four major groups, viz. *Danio*, a monotypic genus with *D. dangila* (Hamilton, 1822) as the type; *Celestichthys* Roberts, 2007 with four especially beautiful tiny

'*Danio*' species with *C. margaritatus* Roberts, 2007 as the type (Roberts, 2007); *Brachydanio* Weber et de Beaufort, 1916 with 10 '*Danio*' species with *B. albolineata* (Blyth, 1860) as the type (Weber & de Beaufort, 1916); and *Devario*, the biggest genus within '*Danio*' groups with 24 valid species with *D. devario* (Hamilton, 1822) as the type. There are totally 8 valid '*Danio*' species in China and they all occur in Yunnan (Chen, 2013). We follow Kottelat (2013) for classification of Chinese Danios here except his tentative assignment of *D. shanensis* (Hora, 1928) into *Inlecypris* Howes, 1980. With the exception of *D. albolineata* moved into *Brachydanio*, all the other seven Chinese species were moved into *Devario*.

From April to June 2015, in field surveys of ichthyofauna in the main stem of Lancangjiang (upper Mekong), Yunnan Province, several new specimens to China were collected (Fig. 1). Further identification confirmed the two species, *Yasuhikotakia lecontei* and *Brachydanio rosea* (Fang et Kottelat, 2000) to be new records to China. Hereinto, is reported, for the first time, a true member of genus *Yasuhikotakia* in China, because the former recorded *Y. nigrolineata* (Kottelat et Chu, 1987) was moved into genus *Ambastaia* Kottelat, 2012 by Kottelat (2012) and followed by Chen (2013). The occurrence of the two species in Xishuangbanna, Yunnan Province is most likely a natural extension of their distributional range.

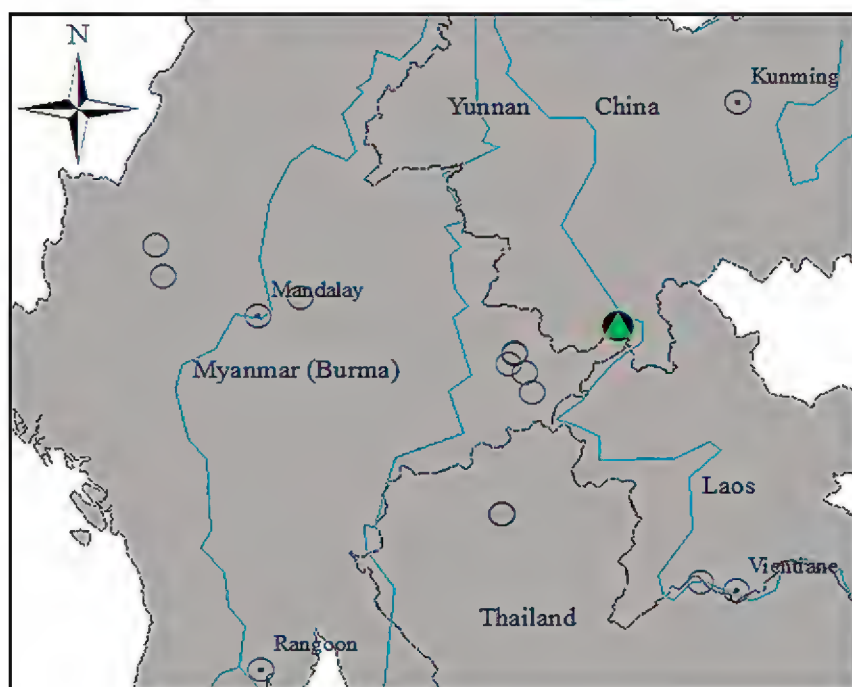


Figure 1. Collecting sites of *Yasuhikotakia lecontei* and *Brachydanio rosea*, data from Fishbase (Froese & Pauly, 2016). ○: City; △: Locality of *Y. lecontei*; ▲: New record of *Y. lecontei*; ○: Locality of *B. rosea*; ●: New record of *B. rosea*.

MATERIAL AND METHODS

Meristics and measurements for *Yasuhikotakia* follow Kottelat (2004), while those for *Brachydanio rosea* follow Fang (1997) and Fang & Kottelat (2000). All measurements were made with a dial caliper and an accuracy of 0.1 mm. Counts were taken under Leica S60 microscope on the left side of specimens. Kubtec-Xpert 80 Digital Cabinet X-ray System is used for vertebra count. Vertebrae include the first four vertebrae which constitute the Weberian organ. GPS coordinates were obtained from Garmin e Trex handheld device. The specimens examined were deposited in 75% alcohol in the repository collection of the Kunming Institute of Zoology (KIZ), the Chinese Academy of Sciences (CAS). ABBREVIATIONS. BD: body depth; BW: body width; ex.: examined specimens; SL: standard length; TL: total length; HL: head length.

RESULTS

Yasuhikotakia lecontei (Fowler, 1937)

Botia lecontei - Fowler, 1937: 125-264

Yasuhikotakia lecontei - Nalbant, 2002: 309-333

EXAMINED MATERIAL. *Yasuhikotakia lecontei*, KIZ2015006371-6373, 3 ex., 54.4-56.4 mm SL (Table 1), from the main stem of Lancangjiang, Wenquan and Mengbo Village, Menghan Township, Jinghong City, Xishuangbanna Prefecture, Yunnan Province, China (21.839542° N, 101.042860° E, 526 m and 21.823279° N, 101.055848° E, 526 m), collected by Wansheng Jiang, Shusen Shu and Congwen Shi, 21 April and 13 June 2015, respectively.

DESCRIPTION. The examined specimen matches well diagnoses of *Y. lecontei* based on its original description (Fowler, 1937) and Kottelat (2001): 9 branched dorsal-fin rays (7-9 in original description) and 3 simple rays; pelvic fin almost reaching anus; suborbital spine bifid, curvulate, its tip of the long spine extending to posterior margin of eye and the short one reaching midpoint of eye, erect ethmoid spine parallel to horizontal axis of eye; relatively large mental lobe; a pair of papillae in lower jaw; a large blackish blotch on caudal peduncle, not extending to upper edge; incomplete lateral line (Fig. 2); total vertebrae 35 (Fig. 3).

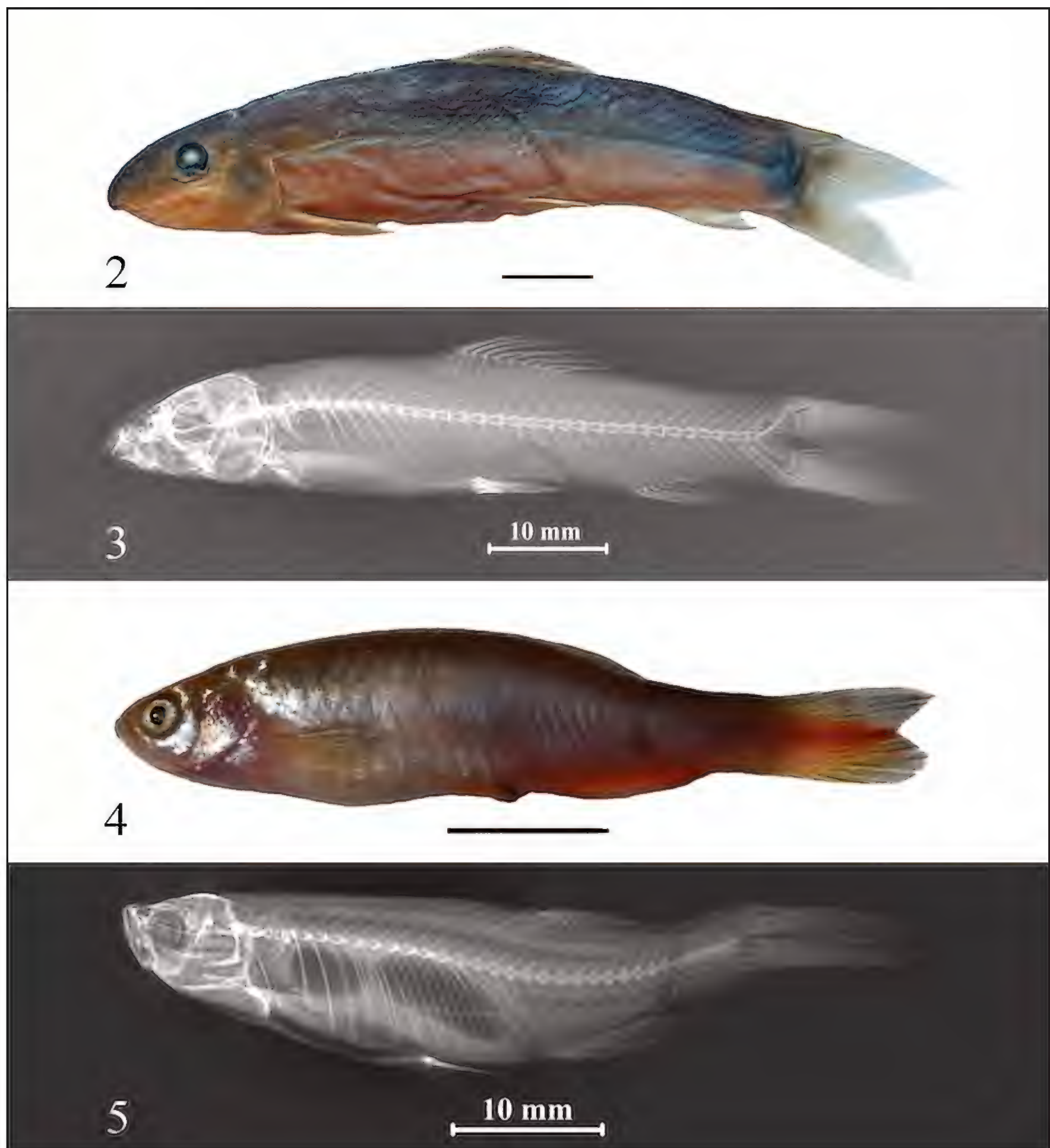


Figure 2. *Yasuhikotakia lecontei*, KIZ2015006373, 56.4 mm SL. Scale bar=1cm. Figure 3. X-ray photograph of *Y. lecontei*, KIZ2015006373, 56.4 mm SL. Figure 4. *Brachydanio rosea*, KIZ2015006374, 41.7 mm SL, shortly after capture (photograph by Wansheng Jiang). Scale bar=1cm. Figure 5. X-ray photograph of *B. rosea*, KIZ2015006374, 41.7 mm SL.

DISTRIBUTION AND BIOLOGY. Before this survey was undertaken, this species was just known in a broad region of Mekong and Chao Phraya basins of Southeast Asia except China, and now it expands to lower Lancangjiang, Yunnan, China (Fig. 1). *Yasuhikotakia lecontei* occurs in flowing rivers with stony to rocky substrate, usually inhabiting in

crevices or cover of rocks in the underwater during the day, going out to foraging at dusk and night; feeding on benthic invertebrates and mollusks; owing to its ornamental value, it is commonly found in the freshwater aquarium trade (Fowler, 1937; Vidthayanon et al., 1997; Kottelat, 1998; 2012; 2013).

Measurements	KIZ2015006371	KIZ2015006372	KIZ2015006373	Range
SL (mm)	55.0	54.4	56.4	54.4-56.4
TL (mm)	67.6	66.8	70.8	66.8-70.8
Percent (%) of SL				
HL	27.1	24.3	24.3	24.3-27.1
Predorsal length	47.6	48.0	50.5	47.6-50.5
Prepelvic length	54.2	54.0	54.6	54.0-54.6
Preanal length	76.9	76.2	78.2	76.2-78.2
BD	23.1	23.9	18.8	18.8-23.9
BW	6.5	6.6	6.9	6.5- 6.9
Caudal-peduncle depth	11.8	11.8	11.7	11.7-11.8
Caudal-peduncle length	13.5	12.3	13.5	12.3-13.5
Percent (%) of HL				
Snout length	43.6	48.7	50.0	43.6-50.0
Eye diameter	20.8	20.5	19.7	19.7-20.8
Interorbital width	37.5	34.8	37.2	34.8-37.5

Table 1. The morphological measurements of *Yasuhikotakia lecontei*.

Measurements	KIZ2015006374
SL (mm)	41.7
Vertebrae	34
Percent (%) of SL	
BD	25.6
HL	20.1
Eye diameter	5.8
Interorbital width	9.7
Caudal-peduncle length	18.1
Caudal-peduncle depth	10.9
Predorsal length	64.6
Preanal length	71.5
Prepelvic length	50.3
Prepectoral length	23.9

Table 2. The morphological measurements of *Brachydanio rosea*.

Brachydanio rosea (Fang et Kottelat, 2000)

Danio roseus Fang et Kottelat, 2000: 149–154
Brachydanio rosea Kottelat, 2013: 1–663.

EXAMINED MATERIAL. *Brachydanio rosea*, KIIZ2015006374, 1 ex., 41.7 mm SL (Table 2), from Lancangjiang basin, Mengbo Village, Menghan Township, Jinghong City, Xishuangbanna Prefecture, Yunnan Province, China (21.823279° N, 101.055848° E, 526 m), collected by Wansheng Jiang, Shusen Shu and Congwen Shi, 13 June 2015.

DESCRIPTION. The examined specimen is exclusively identified as *D. roseus* according to its original description (Fang & Kottelat, 2000) and Kottelat (2001): a small sized species; incomplete lateral-line, perforating 9 scales (vs. 9-10); without infraorbital process; having 7 branched dorsal-fin

rays and 12 branched anal-fin rays (vs. 12-14); having two pairs of well-developed barbels, the rostral barbels extending to the posterior margin of orbit and the maxillary barbels nearly reaching anal-fin origin; body lacking horizontal black stripes along posterior part side; total vertebrae 34 (Fig. 5). Colour in life. Body bluish silver, caudal-fin with bright red median rays and black upper and lower margins, anal fin with a bright red sub-proximal stripe, pelvic fin proximal half orange (Fig. 4).

DISTRIBUTION AND BIOLOGY. *Brachydanio rosea* is usually found in small streams with relative cold running water under forest cover in Mekong basin of northern Thailand, Laos and Myanmar (Fang & Kottelat, 2000; Kottelat, 2001). Its occurrence in the downstream of Lancangjiang Basin in Yunnan, China is not surprising (Fig. 1).

DISCUSSION AND CONCLUSIONS

The herein study reported the occurrence of two new species, *Y. lecontei* and *B. rosea*, in Xishuangbanna Autonomous Prefecture, Yunnan Province. This raises a new genus and two species for the number of freshwater fishes in China. Although there is no opportunity to inspect the types of *Y. lecontei* and *B. rosea*, comparing all counts and measurements with the original descriptions, we found our specimens match well with *Y. lecontei* and *B. rosea*, respectively. Our location of this discovery is in the north-most geological range of these two species in Mekong River Drainage, what reported in the present paper expands the northern boundaries of the distribution of *Y. lecontei* and *B. rosea* and adds a new genus and two species for China records.

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A new species from the Mediterranean Sea and North-Eastern Atlantic Ocean: *Knoutsodonta pictoni* n. sp. (Gastropoda Heterobranchia Nudibranchia)

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ABSTRACT

Knoutsodonta pictoni n. sp. (Gastropoda Heterobranchia Nudibranchia) is described here based on morphological and molecular analyses of specimens from Mediterranean Sea and North Atlantic Ocean. Ecological notes on the egg development and new species distribution range are also presented. COI DNA barcoding was used to molecularly identify this species and to assess one sequence present in Genbank but for which identification was not provided. Furthermore, the intraspecific genetic divergence was explored for specimens belonging to different populations.

KEY WORDS

DNA-Barcoding; new species; Nudibranchs; Onchidorididae; *Knoutsodonta*.

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INTRODUCTION

At the beginning of 2015 the genera placed within the family Onchidorididae (Gastropoda Heterobranchia Nudibranchia) were: *Acanthodoris* Gray, 1850, *Adalaria* Bergh, 1878, *Calycidoris* Abraham, 1876, *Corambe* Bergh, 1869, *Diaphorodoris* Iredale et O'Donoghue, 1923, *Onchidoris* Blainville, 1816 and *Onchimira* Martynov, Korshunova, Sanamyan et Sanamyan, 2009. Later on, Hallas & Gosliner (2015) based on the results of molecular and morphological analyses reestablished the families Corambidae, with the genus *Corambe*, and Calycidorididae including the genera *Calycidoris* and *Diaphorodoris*. The remaining genera were maintained as genus as in the case of the *Acanthodoris*, or divided into two different genera as happened for *Onchidoris* and *Adalaria*. In particular, the species of *Onchidoris* and *Adalaria* with a rachidian tooth

were grouped into the genus *Onchidoris* whereas species with no rachidian tooth were placed in *Knoutsodonta* Hallas et Gosliner, 2015. In 2015, almost concurrently, specimens of the *Onchidoris*-like group were photographed and collected in Sardinia (Italy), in Catalunya (Spain) and in Ireland. Some photographed individuals, displayed in social media, showed a strong similarity with specimens from Mediterranean Sea and North-Eastern Atlantic Ocean.

In Trainito & Doneddu (2015) the Sardinian findings were reported as *Onchidoris* sp. and in the discussion the possibility was expressed that they should be assigned to a new species, considering that their external morphology did not match any of the described species of the family Onchidorididae. One specimen was found at East Wall Loch Nevis, Scotland on 22nd August 2015 and afterwards published by J. Anderson on the web as *Knoutsodonta*

sp. A. (<http://www.nudibranch.org/Scottish%20Nudibranchs/html/knoutsodonta-spA-01.html>).

A similar specimen was photographed in 2011 by G. Brown at Loch Sween, Scotland and published by M. Faasse on the Facebook group page of NE Atlantic Nudibranchs. Subsequently, in Ballesteros et al. (2016), one photograph of an individual was reported showing the same external features of the samples found in 2015. In the discussion, it was described as *Knoutsodonta* sp. A, based on a preliminary analysis of the radula that lacked the rachidian tooth, and the dataset of observations was increased, dating back to 1992. In 2016 other individuals with the same external morphology were photographed and collected in Northern Adriatic Sea (Sistiana, Trieste, Italy). One individual with the characteristic egg coils, was found at Cape Noli (Liguria, Italy) and preliminarily identified as *Onchidoris pusilla* (Alder et Hancock, 1845) (Betti et al., 2017), but the external morphology revealed this to be more similar to the specimens reported in

Trainito & Doneddu (2015). Furthermore, other specimens with the same phenotype have been reported as *Onchidoris pusilla* from Ensenada de los Berengueles (Granada, Spain), Estarrit, Blanes and Tossa de Mar (Catalunya, Spain) (GROC, <http://www.opistobranquis.org/en/guia/10>).

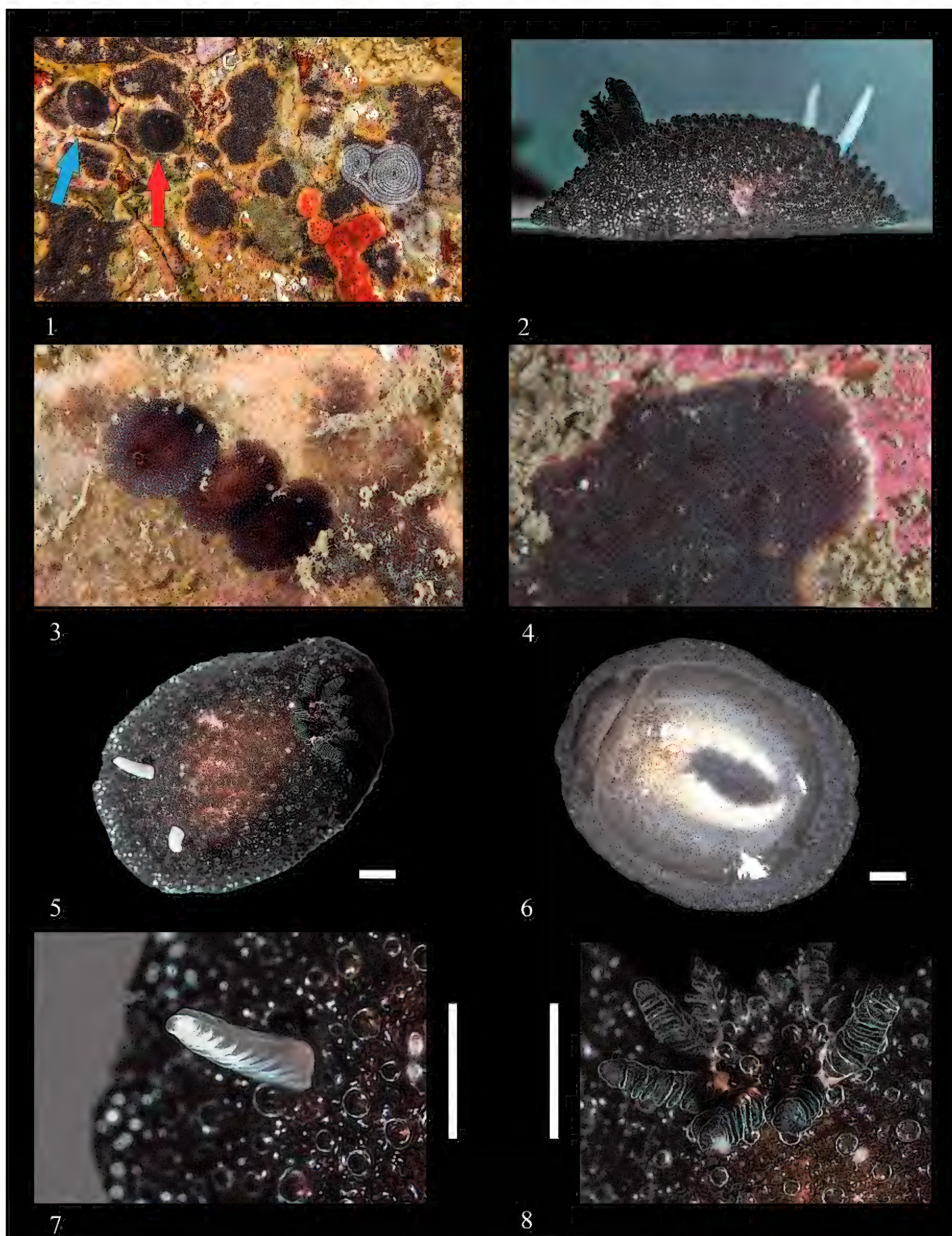
In this paper we describe this species as new to science, through morphological and molecular analyses on individuals collected from Central Tyrrhenian (North-Eastern Sardinia) and Adriatic (Trieste) Seas and from the North-eastern Atlantic Ocean (Ireland), discussing their taxonomic position with a focus on other Mediterranean species of the genus.

MATERIAL AND METHODS

Specimens of the new species were collected by scuba diving from different localities (Table 1). Individuals, egg spawns and the bryozoans on which

Species	Voucher	Locality	COI accession number	References
<i>Knoutsodonta brasiliensis</i>	BNHS-Opistho-336	-----	KC255226	Bhave et al., unpublished
<i>Knoutsodonta depressa</i>	CASIZ186769A	Huelva, Spain	KP340409	Hallas & Gosliner, 2015
<i>Knoutsodonta jamnae</i>	CASIZ175578	Pillar Point: San Mateo Co., California	KP340392	Hallas & Gosliner, 2015
<i>Knoutsodonta oblonga</i>	MN3010A	Mewstone, Skomer	KP340410	Hallas & Gosliner, 2015
<i>Knoutsodonta pictoni</i>	BAU02982	South of Inishgalloon, Purteen, Keel, Achill Island, Ireland	LT840347	Present study
<i>Knoutsodonta pictoni</i>	BAU02983	South of Inishgalloon, Purteen, Keel, Achill Island, Ireland	LT840348	Present study
<i>Knoutsodonta pictoni</i>	BAU02985	Sistiana, Trieste, Italy	LT840346	Present study
<i>Knoutsodonta pictoni</i>	MNHN-IM-2000-33333	Porto San Paolo, Sardinia, Italy	LT840344	Present study
<i>Knoutsodonta pictoni</i>	MNHN-IM-2000-33334	Porto San Paolo, Sardinia, Italy	LT840345	Present study
<i>Knoutsodonta</i> sp. A	CASIZ 208194	Scotland	KP340411	Hallas & Gosliner, 2015
<i>Onchidoris bilamellata</i>	MT09252	North Sea	KR084801	Barco et al., 2016
<i>Onchidoris bilamellata</i>	CASIZ_101555	Peninsula Point, Marin Co., California	KP340408	Hallas & Gosliner, 2015
<i>Onchidoris bilamellata</i>	10NBMOL-10020	Passamaquoddy Bay, St. Andrews, New Brunswick, Canada	KF644026	Layton et al., 2014
<i>Onchidoris bilamellata</i>	10NBMOL-10019	Passamaquoddy Bay, St. Andrews, New Brunswick, Canada	KF643873	Layton et al., 2014
<i>Onchidoris bilamellata</i>	10BCMOL-00203	Rocky beach, Wizard Islet, Bamfield, British Columbia, Canada	KF643475	Layton et al., 2014
<i>Onchidoris bilamellata</i>	10NBMOL-10018	Passamaquoddy Bay, St. Andrews, New Brunswick, Canada	KF643245	Layton et al., 2014
<i>Onchidoris evincta</i>	CASIZ_187758B	Puget Sound, Kitsap Co., Washington	KP340391	Hallas & Gosliner, 2015
<i>Onchidoris evincta</i>	CASIZ_186817	Puget Sound, Kitsap Co., Washington	KP340389	Hallas & Gosliner, 2015
<i>Onchidoris loveni</i>	NTNU_65511B	Mausund, Norway	KP340395	Hallas & Gosliner, 2015
<i>Onchidoris loveni</i>	NTNU_65511A	Mausund, Norway	KP340394	Hallas & Gosliner, 2015
<i>Onchidoris loveni</i>	NTNU_66880A	Gløssvika, Norway	KP340393	Hallas & Gosliner, 2015
<i>Onchidoris muricata</i>		Clachan Seil, Scotland	AY345033	Grande et al., 2004
<i>Onchidoris muricata</i>	MT07703	North Sea	KR084489	Barco et al., 2016
<i>Onchidoris muricata</i>	CASIZ_184185A	New Castle Portsmouth Bay: Rockingham Co., New Hampshire	KM219681	Hallas & Gosliner, 2015
<i>Onchidoris muricata</i>	CASIZ_181312	Asilomar, Monterey Co., California	KM219680	Hallas & Gosliner, 2015
<i>Onchidoris muricata</i>	10BCMOL-00318	Juskalta Narrows, Haida Gwaii, British Columbia, Canada	KF643468	Layton et al., 2014
<i>Onchidoris muricata</i>		Kristineberg, Bohuslan, Sweden	AJ223271	Tholleson M., 2000
<i>Onchidoris proxima</i>	CASIZ_183931A	Passamaquoddy Bay Eastport: Washington Co., Maine	KM219677	Hallas & Gosliner, 2015
<i>Onchidoris proxima</i>	CASIZ_183921A	Passamaquoddy Bay Eastport: Washington Co., Maine	KM219676	Hallas & Gosliner, 2015
<i>Diaphorodoris luteocincta</i>	BAU2754	Gulen, Norway	LT615386	Furfaro et al., 2016
<i>Diaphorodoris luteocincta</i>	BAU2755	Gulen, Norway	LT615387	Furfaro et al., 2016
<i>Diaphorodoris luteocincta</i>	BAU2756	Gulen, Norway	LT615388	Furfaro et al., 2016

Table 1. List of the species names, vouchers, collection localities, COI GenBank accession numbers and references of the species of *Knoutsodonta* and *Onchidoris* genera and the out-group.



Figures 1–4. In situ photographs of the living specimens of *Knoutsodonta pictoni* n. sp. Fig. 1: holotype (MNHN-IM-2000-33333) (red arrow) and paratype (MNHN-IM-2000-33333) (light blue arrow) and the eggs (on the right side). Fig. 2: lateral view of the *K. pictoni* n. sp. Sardinian specimen (MNHN-IM-2000-33333). Fig. 3: three adults specimens from Ireland. Fig. 4: the encrusting bryozoan *Reptadeonella violacea* on which the new nudibranch species feed on. Figures 5–8. Holotype of the *Knoutsodonta pictoni* n. sp. (MNHN-IM-2000-33333). Fig. 5: dorsal view of the living adult. Fig. 6: ventral view. Fig. 7: a particular of the shape of the rhinophores. Fig. 8: a particular of the gills. Scale bar = 1 mm.

they were feeding were documented in situ with high definition photographs. Sardinian specimens were kept in an aquarium where both egg deposition and development were documented up to the veliger stage. The holotype and the paratype of the new species were preserved in Ethanol 95% and deposited at the Muséum national d'histoire naturelle (MNHN). All other collected individuals were stored in the malacological collection at the Department of Biology and Biotechnologies “Charles Darwin” (“Sapienza” University of Rome, Italy) (Table 1).

Anatomy of the reproductive system was studied under a dissecting microscope from at least two individuals. The buccal mass was placed in a 10% NaOH solution to isolate the radula, which was dehydrated to 100% ethanol, critical point-dried, gold coated, and examined by a Dualbeam SEM. The reproductive system was observed under a dissecting optical microscope and photographed at different stages of dissection.

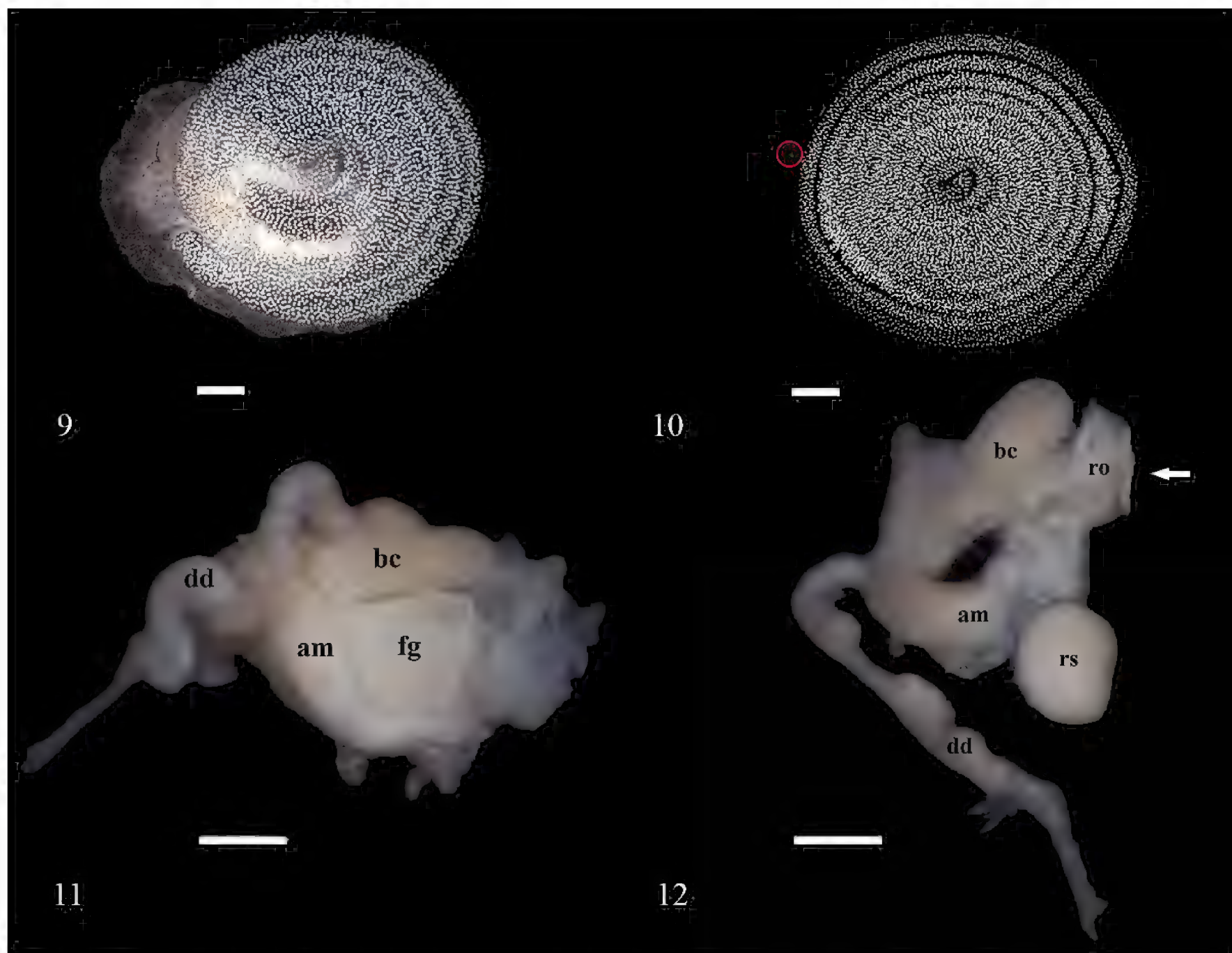
Morphological analyses of the radula structure were performed using both SEM and optical microscope techniques.

Molecular identity was tested by using a partial sequence of the molecular marker mostly used for DNA barcoding of nudibranchs, the mitochondrial cytochrome c oxidase subunit I (COI) (see Table 1 for full list of samples, localities, and voucher references). A piece of tissue was cut from the foot for DNA extraction. Total genomic DNA was extracted using a standard proteinase K phenol/chloroform method with ethanol precipitation, as reported in Oliverio & Mariottini (2001). Partial sequences of COI were amplified by polymerase chain reaction (PCR) using the primers LCO1490(5'-GGTCAA-CAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAATCA-3') (Folmer et al., 1994) (PCR profile: 5 min denaturation step at 94°C; 35 cycles of 94°C/30 s, 48°C/60 s, 72°C/60 s; 7 min final extension at 72°C). Amplicons were sequenced by European Division of Macrogen Inc. (Amsterdam, The Nederland), using the same PCR primers. Sequences from each DNA strain were assembled and edited with Staden Package 2.0.0b9 (Staden et al., 2000). BLASTN (Altschul et al., 1990) search was conducted to exclude contamination. Sequences obtained were aligned together with those already present in GenBank using Muscle algorithm implemented in MEGA 6.0 (Tamura et al., 2013). We employed the

Automatic Barcode Gap Discovery (ABGD, available at <http://www.wabi.snv.jussieu.fr/public/abgd/>), a distance-based method designed to detect the so called “barcode gap” in the distribution of pairwise distances calculated in a sequence alignment (Puillandre et al., 2012a, b) to test species delimitation in the newly produced COI dataset and produce primary species hypotheses based on DNA distances. Alignment of the COI sequences was submitted and processed in ABGD using the Kimura two parameters (K2p) nucleotide substitution model and the following settings: a prior for the maximum value of intraspecific divergence between 0.001 and 0.1, 30 recursive steps within the primary partitions defined by the first estimated gap, and a gap width of 0.1. The uncorrected pairwise genetic distances (p-distance) among COI sequences of the putative new species were also calculated with MEGA 6.0, and the maximum intraspecific distance estimated. Phylogenetic analyses on the COI dataset, were then performed basing on Bayesian Inference (BI) and Maximum Likelihood (ML) methods to test whether the primary species hypotheses of ABGD proved also monophyletic. In particular, BI was performed using MrBayes 3.2.6 (Ronquist et al., 2012) with four Markov-chains of five million generations each, sampled every 1000 generations. Consensus trees were calculated on trees sampled after a burnin of 25%. ML searches were performed using MEGA 6.0 with a starting tree topology generated by a Neighbour Joining algorithm (Zwickl, 2006). Nodal support was assessed by means of 1000 bootstrap replicates. The model of evolution was selected in JModel Test 0.1 (Posada, 2008) according to the Bayesian Information Criterion (BIC). *Diaphorodoris luteocincta* (Voucher BAU2754) was used as out-group since it shows a basal placement within Onchidoridoidea according to Hallas & Gosliner (2015).

RESULTS AND DISCUSSION

Ten individuals were observed in situ (Figs. 1–4) and, within these, six were collected, from different localities (Table 1), and studied in an aquarium (Figs. 5–8) before anatomical analysis. The egg development was documented until the veliger stage (Figs. 9, 10).



Figures 9–12. *Knoutsodonta pictoni* n. sp. Fig. 9: in aquarium eggs deposition. Fig. 10: eggs at different developmental stages. One veliger is indicated with a red circle. Figs. 11, 12: reproductive system: bc = bursa copulatrix, ro = reproductive opening, dd = deferent duct, am = ampulla, fg = female gland, rs = receptaculum seminis. Scale bar = 1 mm.

Diagnostic anatomical features were compiled from at least two specimens (Figs. 11, 12; Figs. 13–18).

DNA-barcoding. The COI sequences were deposited at the European Nucleotide Archive (<http://www.ebi.ac.uk/ena>) and the information on voucher, accession numbers and collection localities are listed in Table 1. The BLAST search found a high similarity (99%) between the new species sequences and one sequence present in GenBank (Accession number: KP340411, voucher: CASIZ 208194) identified as *Knoutsodonta* sp. A. The pairwise genetic distances calculated on the COI sequences revealed 2.0% of maximum intraspecific divergence of the putative new species (Table 2). A final COI dataset, excluding the out-group, consisted of 29 sequences from ten different species belonging to *Knoutsodonta* and *Onchidoris*. The

COI final alignment consisted of 609 nucleotide positions with 198 polymorphic sites.

Results from the ABGD analysis returned 11 Preliminary Species Hypothesis (PSH) with *O. bilamellata* sequences split in two different PSH. All the recursive steps in the ABGD analysis resulted in the same sequence repartition, with all the sequences of the putative new species grouped in a single PSH (Fig. 19) including *Knoutsodonta* sp. A (KP340411).

The phylogenetic analyses resulted in monophyletic clades (Fig. 20) that were congruent with the PSH obtained with the ABGD analysis. The phylogenetic inference confirmed that all the specimens belonging to the putative new species grouped in one monophyletic clade together with the GenBank sequence of *Knoutsodonta* sp. A (KP340411) with high support values (BI=1, ML=100). The

COI resulting topology showed also that the sequences ascribed to *O. bilamellata* split in two divergent clades congruently with the ABGD species delimitation results. In particular, the specimen of '*O. bilamellata*' with COI accession number KR084801, was the sister to the new species, with high statistical support (BI=1, ML=99).

TAXONOMY

Familia ONCHIDORIDIDAE Gray, 1827

Genus *Knoutsodonta* Hallas et Gosliner, 2015

Knoutsodonta pictoni n. sp. (Figs. 1–8)

TYPE MATERIAL. Holotype. Voucher MNHN-IM-2000-33333, 11 mm in length alive, Tavolara-Punta Coda Cavallo Marine Protected Area (MPA), Porto San Paolo, North Eastern Sardinia, Central Tyrrhenian Sea, Mediterranean Sea, Italy, (40.881635°N, 9.637065°E), 31.III.2015 (Figs. 1, 2, 5–8).

Paratype. Voucher MNHN-IM-2000-33334, one specimen, dissected, 10 mm in length alive, same data as the holotype (Fig. 1).

OTHER EXAMINED MATERIAL. BAU02982, one specimen, dissected, 8 mm in length alive, South of Inishgalloon, Purteen, Keel, Achill Island, Ireland, Atlantic Ocean, (53.9556°N, 10.1023°W), 05 April 2015 (Fig. 3). BAU02985, one specimen, 9 mm in length alive, 6 m depth, Sistiana, Trieste, North Adriatic Sea, Mediterranean Sea, Italy, (45.7728726°N, 13.6292581°E), 31 December 2016. BAU02983, one specimen, 6 mm in length alive, South of Inishgalloon, Purteen, Keel, Achill Island, Ireland, Atlantic Ocean, (53.9556°N, 10.1023°W), 05 April 2015 (Fig. 3). BAU02984, one specimen, 6 mm in length alive, South of Inishgalloon, Purteen, Keel, Achill Island, Ireland, Atlantic Ocean, (53.9556°N, 10.1023°W), 05 April 2015 (Fig. 3).

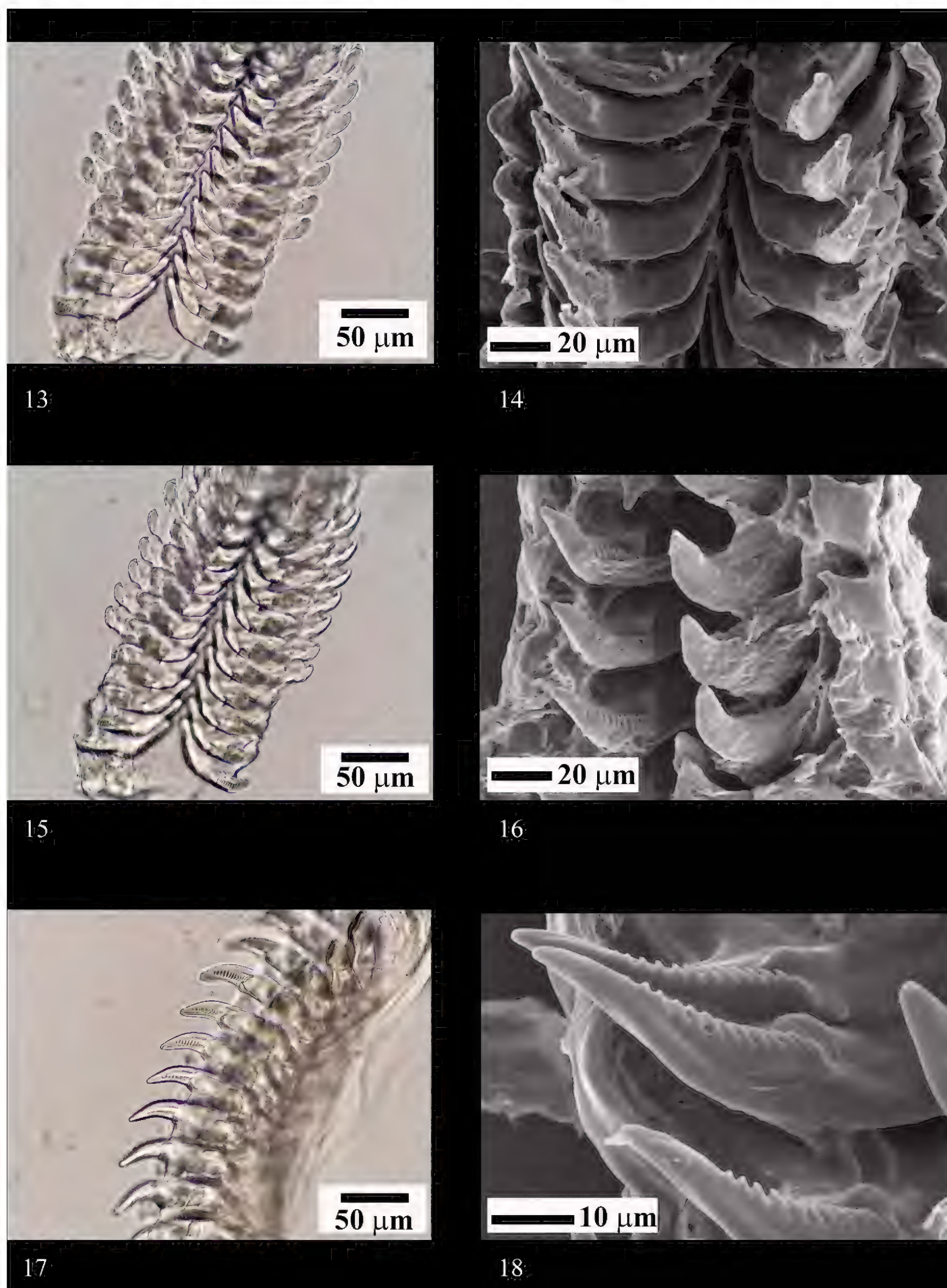
DESCRIPTION OF HOLOTYPE. External morphology: body elliptical, depressed, equally rounded both in anterior and posterior edges. Body colour uniform dark brown, almost black with small bluish white speckles scattered along the mantle (Figs. 1, 5). Body colour of ventral part light grey with diffuse spots dark brown as mantle (Figs. 5, 6). Long and slender rhinophores, totally white, retractable,

lamellate (10 lamellae) (Fig. 7). Foot not projected beyond notum: anterior margin indented forming two large lobes, posterior part forms a rounded tail (Fig. 6). Mantle structure very hard, with strong reticulation formed by translucent spicules. Almost globular tubercles, evenly spaced, covering mantle and surrounding anus opening and rhinophores sheaths. Mouth surrounded by large oral veil with just outlined lateral lobes (Fig. 6). Non-retractile gills with 9 bipinnate branchial leaves, larger anteriorly than posteriorly, forming an almost complete semicircle around the anus: one tubercle may be present just behind the anus (Fig. 8). Several narrow tubercles of variable height inside the gill circlet.

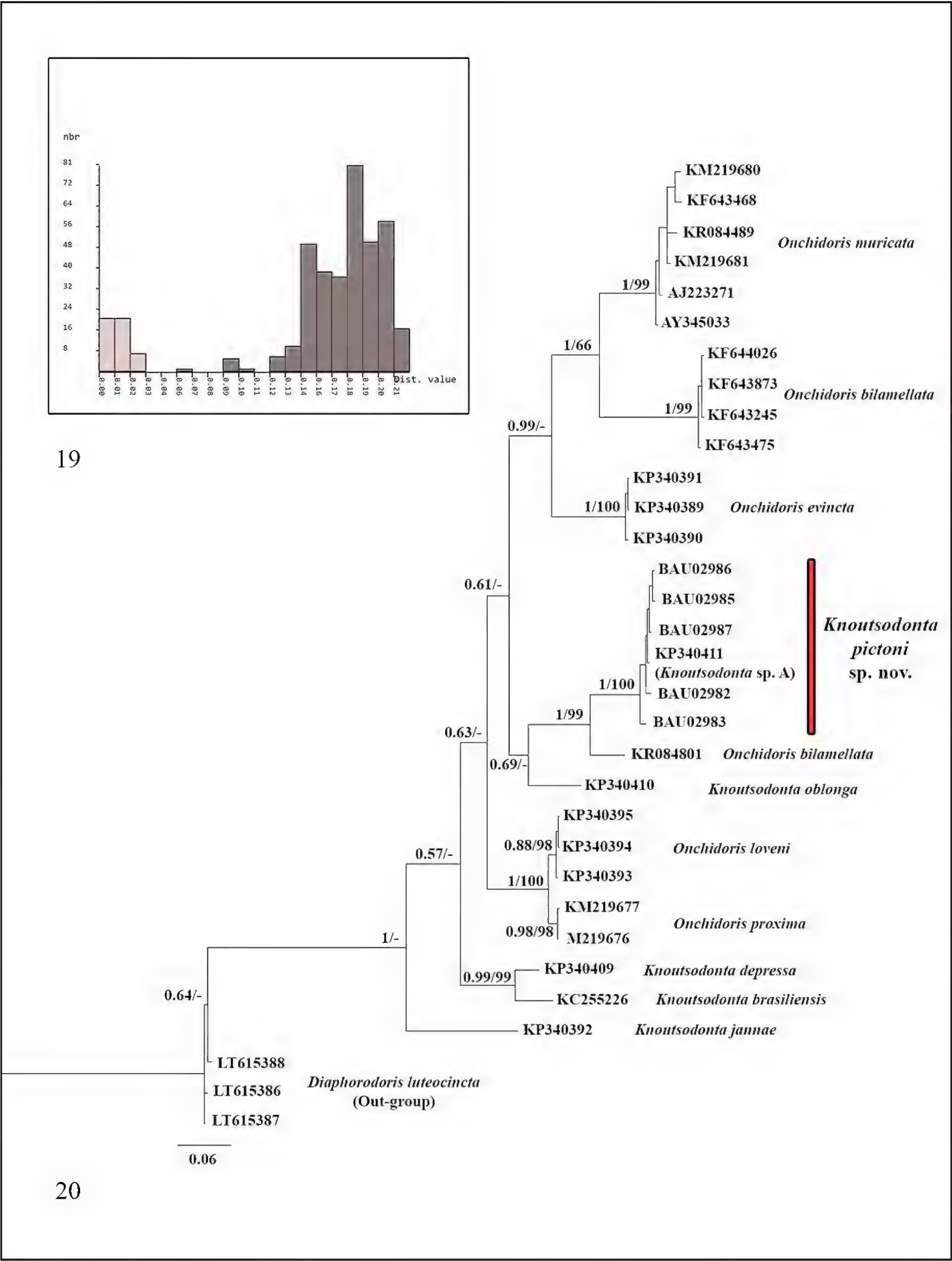
VARIABILITY. Body length ranging from 9 mm to 12 mm (10 specimens examined). Rhinophores with 9–11 lamellae. Gills with 9 or 10 bipinnate branchial leaves.

Paratype internal anatomy: Rachidian tooth absent. Radular formula 25–28 x 1.1.0.1.1. Radular teeth almost colourless (Figs. 13, 14). First lateral teeth with long, wide base and strong, almost straight beak-shaped cusp, denticulate with 10–13 small denticles along the internal surface (Figs. 15–18). Second lateral teeth in shape of rectangular plates, with downward directed cusp on lower outside corner (Figs. 15, 16). Swollen tube of ampulla connected through a short duct to bursa copulatrix and seminal male duct. Bursa copulatrix leads to distal part of female duct, in connection with seminal receptaculum ending into vagina and female opening. Relatively long loop of prostatic part of vas deferens adjacent to bursa copulatrix (Figs. 11, 12). Prostate smooth, not granulated, first narrow, then rapidly wide into a long swollen penial sheath with several folds of ejaculatory duct. (Figs. 11, 12). Bean-shaped bursa copulatrix, slightly yellowish (Figs. 11, 12), enters distal part of vagina through a relatively long stalk. At its base, a duct leads to ovoid seminal receptacle (Figs. 11, 12). Vagina wide and long (Figs. 11, 12).

ETYMOLOGY. The new species is named after Bernard Picton ((National Museums Northern Ireland, 153 Bangor Road, Cultra, Holywood, BT18 0PE, UK)) who kindly presented us specimens from Ireland and whose prominent work on North Atlantic nudibranchs is a mandatory reference for researchers and nudibranch enthusiasts all over the world.



Figures 13–18. Radula of the *Knoutsodonta pictoni* n. sp. Figs. 13, 15, 17: Optical microscope images. Figs. 14, 16, 18: SEM images at different magnification levels.



Figures 19, 20. DNA Barcoding analyses. Fig. 19: the ABGD histogram of the COI barcoding region shows the distribution of the pairwise estimated genetic distances (K2p) in intraspecific (left, light grey) and interspecific (right, dark grey) comparisons. Fig. 20: Bayesian resulting tree of the COI dataset. Numbers at nodes are Bayesian posterior probability and ML bootstrap support, respectively.

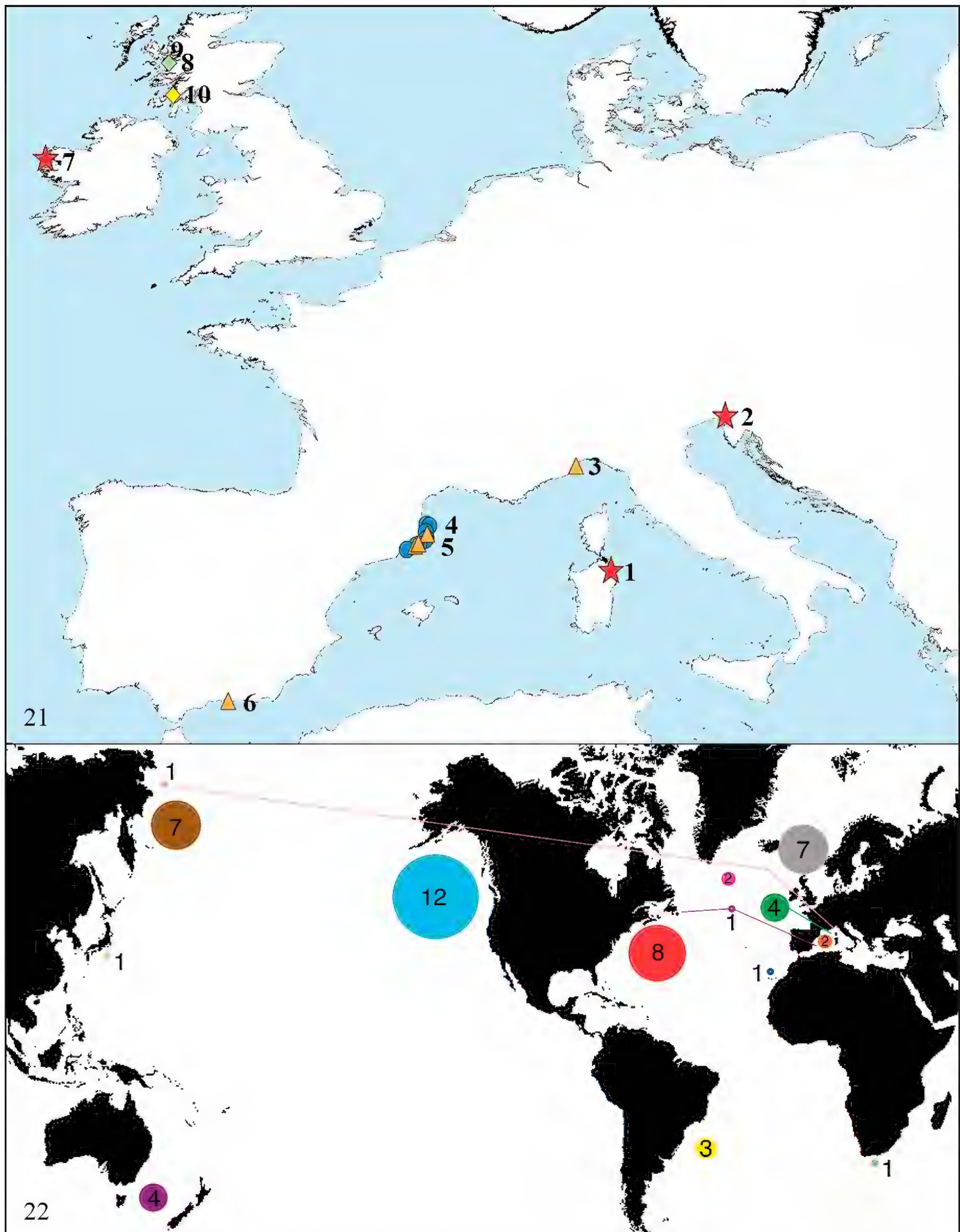


Figure 21. Distribution of *Knoutsodonta pictoni* n. sp. The black numbers correspond to the sites indicated in Table 3. Specimens examined in the present paper, red star; Atlantic specimen identified as *Knoutsodonta* sp. A (KP340411), green lozenge; Atlantic specimen identified as *Onchidoris* sp., yellow lozenge; Mediterranean specimens identified as *Onchidoris pusilla*, orange triangle; Mediterranean specimens identified as *Knoutsodonta* sp. A, blue circle. Figure 22. Distribution of the species belonging to the family Onchidorididae. The numbers correspond to the number of species with the same distribution, the size of the circles is correlated to the number of species.

	<i>K. pictoni</i> BAU02986	<i>K. pictoni</i> BAU02987	<i>K. pictoni</i> BAU02985	<i>K. pictoni</i> BAU02982	<i>K. pictoni</i> BAU02983	<i>K. sp. A</i> KP340411
<i>K. pictoni</i> BAU02986	0.000					
<i>K. pictoni</i> BAU02987	0.007	0.000				
<i>K. pictoni</i> BAU02985	0.003	0.007	0.000			
<i>K. pictoni</i> BAU02982	0.015	0.011	0.015	0.000		
<i>K. pictoni</i> BAU02983	0.020	0.016	0.020	0.018	0.000	
<i>K. sp. A</i> KP340411	0.008	0.005	0.008	0.010	0.015	0.000

Table 2. Pairwise distances (p-distance) between specimens belonging to *Knoutsodonta pictoni* n. sp.

<i>Knoutsodonta pictoni</i> n. sp.		
Map sites	Locality	Reference source
1	MPA Tavolara, Sardegna, Italy	Present paper
2	Sistiana, Trieste, Italy	Present paper
3	Cape Noli, Liguria, Italy	as <i>Onchidoris pusilla</i> in Betti et al. 2017. J.Molluscan Stud., 83:325–332 https://doi.org/10.1093/mollus/eyx019
4	Catalunya, Spain	as <i>Knoutsodonta</i> sp. A. in Ballesteros M., Madrenas E. & Pontes M., 2016. Actualización del catálogo de los moluscos opistobranquios (Gastropoda: Heterobranchia) de las costas catalanas. Spira, 6: 1–28.
5	Catalunya, Spain	as <i>Onchidoris pusilla</i> in GROC, 2017. http://www.opistobranquis.org/en/guia/100 .
6	Granada, Spain	as <i>Onchidoris pusilla</i> in GROC, 2017. http://www.opistobranquis.org/en/guia/100 .
7	South of Inishgalloon, Purteen, Keel, Achill Island, Ireland	Present paper
8	Scotland	as <i>Knoutsodonta</i> sp. A, in Hallas J.M. & Gosliner T.M., 2015. Family matters: the first molecular phylogeny of the Onchidorididae Gray, 1827 (Mollusca, Gastropoda, Nudibranchia). Molecular phylogenetics and evolution, 88: 16–2
9	Scotland, East Wall Loch Nevis	as <i>Knoutsodonta</i> sp. A. in http://www.nudibranch.org/Scottish%20Nudibranchs/html/knoutsodonta-spA-01.html .
10	Scotland, Loch Sween	as <i>Onchidoris</i> sp. in NE Atlantic Nudibranch group page posted by M. Faasse on 03/02/2012

Table 3. List of the map sites, localities and reference sources of the records of *Knoutsodonta pictoni* n. sp. Mediterranean Sea, green lines; North Atlantic Ocean, blue lines.

Species attributed to the genus <i>Knoutsodonta</i>					
Species	Body	Rhinophores	Gills	Radular formula	Distribution
<i>Knoutsodonta pictoni</i> n. sp.	Uniform dark brown, almost black with small bluish white speckles scattered along the mantle	White	Dark	25-28 x 1.1.0.1.1	NE Atl. - Med
<i>Knoutsodonta albonigra</i> (Pruvot-Fol, 1951) = <i>Onchidoris albonigra</i>	Translucent white, almost entirely covered by a black texture in relief: at the center of the mantle can be seen the red mass of viscera	White	Whitish	30x1.1.0.1.1	NE Atl. - Med
<i>Knoutsodonta bouvieri</i> (Vayssi��re, 1919) = <i>Onchidoris bouvieri</i>	Pale translucent pink with scattered red brown patches distributed in three longitudinal bands	Yellowish pink	Yellowish pink	40x1.1.0.1.1	Med. endemic?
<i>Knoutsodonta brasiliensis</i> (Alvim, Padula et Pimenta, 2011) = <i>Onchidoris brasiliensis</i>	Greyish white or yellowish orange, both translucent with a pattern of scattered dark brown (sometimes orange) tiny spots, tending towards the mid-line	Translucent white	Translucent white	16-17x1.1.0.1.1	SE Atlantic
<i>Knoutsodonta cervinoi</i> (Ortea et Urgorri, 1979) = <i>Onchidoris cervinoi</i>	Orange, centrally intense, fading at the borders. Some specimens' borders almost whitish	Orange with dark brown leaflets	Dark brown circled by a light area	?x1.0.1	NE Atlantic
<i>Knoutsodonta depressa</i> (Alder et Hancock, 1842) = <i>Onchidoris depressa</i>	Pale or translucent brown with scattered orange or purple-brown speckles	Translucent	Translucent	33-34x1.1.0.1.1	NE Atl. - Med
<i>Knoutsodonta inconspicua</i> (Alder et Hancock, 1851) = <i>Onchidoris inconspicua</i>	White or pale brown, often tinged with a purple hue, covered by small specks of brown pigment	Pale yellow	White	29x1.1.0.1.1	NE Atlantic
<i>Knoutsodonta jannae</i> (Millen, 1987) = <i>Adalaria jannae</i>	Pale yellow or creamy white or orange or lemon yellow	Darker than body	Darker than the body	28-39x4-6.1.0.1.4-6	NE Pacific
<i>Knoutsodonta iannaella</i> (Martyanov, Sanamyan et et Korshunova, 2015) = <i>Adalaria jannaella</i>	Translucent, centrally whitish with a brown patch, central tubercles yellowish, lateral whitish	Yellowish	White	20x2-4.1.0.1.4-2	NE Pacific
<i>Knoutsodonta maugeansis</i> (Burn, 1958) = <i>Onchidoris maugeansis</i>	Pale pattern with shades or colours of yellow and/or orange	Yellowish	Yellowish	22 rows	SW Pacific
<i>Knoutsodonta neapolitana</i> (Delle Chiaie, 1841) = <i>Onchidoris neapolitana</i>	Yellowish or pale brown covered with dense and intensive reddish or purple brown; at the notal edge pigment becomes linear	Dark	Dark	24x1.1.0.1.1	Med endemic
<i>Knoutsodonta oblonga</i> (Alder et Hancock, 1845) = <i>Onchidoris oblonga</i>	Grey with a few darker blotches on the back	Yellowish	Yellowish white	28x1.1.0.1.1	NE Atlantic
<i>Knoutsodonta pusilla</i> (Alder et Hancock, 1845) = <i>Onchidoris pusilla</i>	Dense dark brown pigment spots responsible for dark appearance	Transparent	Transparent	21-29x1.1.0.1.1	NE Atlantic
<i>Knoutsodonta reticulata</i> (Ortea, 1979) = <i>Onchidoris reticulata</i>	Whitish intensely pigmented in green at center, fading at borders; whitish longitudinal bands forming with bands running from tubercles a net surrounding brown green patches	Large brown stains	Pigmented dirt cream	51-54x1.1.0.1.1	NE Atlantic
<i>Knoutsodonta sparsa</i> (Alder et Hancock, 1846) = <i>Onchidoris sparsa</i>	Pale brown mantle with regularly spaced, darker blotches, forming patches	Blotched with olive-brown	Colourless	32-36x1.1.0.1.1	NE Atl. - Med
<i>Knoutsodonta tridactila</i> (Ortea et Ballesteros, 1982) = <i>Onchidoris tridactila</i>	Whitish with orange or dark reddish spots; in between rhinophores and gills up to six irregular lines formed by this pigment	Yellow or light pink	Yellow	12x1-1-0-1-1	NE Atlantic

Table 4. Comparison between external morphology, radular formula and distribution of *Knoutsodonta pictoni* n. sp. with those of the other 14 *Knoutsodonta* species (Mediterranean species in grey lines).

DISTRIBUTION. The species is distributed in Western and Central Mediterranean and in North East Atlantic Ocean. In addition to the localities listed in Table 1 this species has been also recorded under the name of *Knoutsodonta* sp. in different localities along the coast of Catalunya (Ballesteros et al., 2016); it is also recorded in the coast of Granada, in Estartit, in Tossa de Mar and Blanes (Spain) and in Cape Noli (Italy) as *Onchidoris pusilla* (Alder et Hancock, 1845) (GROC, www.opisthobranquis.org, and Betti et al., 2017). The species distribution is summarized in figure 21 and Table 3.

REMARKS. At present, there are 15 Onchidorididae species that can be ascribed to the genus *Knoutsodonta*, based on their radular formula (Hallas & Gosliner, 2015) and among them, five have a Mediterranean distribution (Table 4).

The main morphological features that diagnose *K. pictoni* n. sp. are the dark brown background body colour, the white rhinophores and the dark gills. None of the known Onchidorididae displays these three combined external morphological characters (Table 4). The confusion that has occurred with *O. pusilla* can be easily resolved mainly because this species has translucent white gills and secondly for the body colour whose apparent darkness is due to the presence, on the mantle, of dense dark brown pigmented spots. A comparison of the main morphological features between correlated species of the genus *Knoutsodonta* is reported in Table 4.

All the specimens collected were found feeding and spawning on the encrusting bryozoan *Reptadeonella violacea* (Johnston, 1847) (Gymnolaemata: Chelostomatida: Adeonidae) (Fig. 4), a species of warm temperate waters, distributed in the Mediterranean, and from the North East Atlantic to West Africa waters. The species is also present in West Atlantic (North America and Gulf of Mexico) and along the Pacific Coast of North America (Hayward & McKinney, 2002). When the nudibranchs are upon their bryozoan prey they are very cryptic, and in fact they can be detected only for the presence of a discoloured area of the bryozoan where they have fed (or are feeding) or for the presence of the egg coils. The egg spawn of *K. pictoni* n. sp. is very distinctive among those of the Onchidorididae: it is a flat mucous ribbon forming an almost perfect Archimedean spiral containing egg capsules (Figs. 9, 10). Sometimes two different individuals spawn

in the same place and it may happen that part of the last laid ribbon surrounds the other one (Fig. 1). In aquarium, at 18 °C, one specimen was observed spawning on 6th of April 2015 at 7.00 PM (Fig. 9). On 8th of April at 10.00 AM the coil was full of 9248 egg capsules, while 3 veligers were swimming nearby (Fig. 10). At 8.30 PM of the same day there was only a slight trace of the coil and a large number of veligers was swimming in the entire mass of water.

DISCUSSION

A recent phylogenetic reassessment of the family Onchidorididae by Hallas & Gosliner (2015) proposed some systematic changes regarding the genera *Adalaria*, *Onchidoris* and *Knoutsodonta*. According to this revision, we described a new species of this group through an integrative approach. Morphological evidences revealed the absence of the rachidian tooth in the new species, positioning it in the genus *Knoutsodonta*, while all the molecular analyses performed confirmed the assignment of the Mediterranean and Atlantic specimens to the new species *K. pictoni* n. sp. Additionally, the COI DNA barcoding allowed to identify one sequence present in GenBank (COI accession number KP340411) and corresponding to the new species here described. Interestingly, phylogenetic analyses here proposed (although based on the single COI marker), revealed *K. pictoni* n. sp. as sister to (yet not conspecific with) a specimen (COI accession number KR084801) previously ascribed to *O. bilamellata* that needs further analyses. The study by Hallas & Gosliner (2015) could not provide a complete definition of the genus *Knoutsodonta* and, furthermore, it included only two out of the five Mediterranean species of this genus. The species of Onchidorididae are mainly distributed in the northern hemisphere, and, as depicted in figure 7, for the total of 54 accepted species the highest diversity area is the North Atlantic with the presence of 25 species from the NW Atlantic to the Mediterranean Sea. Twelve species are distributed in the NE Pacific, while eight are in the NW Pacific. Four species are distributed in the SW Pacific, one in Central W Pacific, three in SW Atlantic and one in SE Atlantic-S Indian Ocean.

With the description of *K. pictoni* n. sp., the Onchidorididae of the Mediterranean Sea raise to 8 species, two of which endemic, whose generic attribution needs further investigation. For all these reasons a further integrative study is desirable to understand the systematic position of some critical Onchidorididae taxa and to investigate on the validity of the genus *Knoutsodonta*.

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A Taxonomic study of the Pygmygrasshopper (Orthoptera Tetrigidae) from India with description of a new species

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ABSTRACT

A new species of genus *Coptotettix* Bolivar, 1887, *C. magedhensis* n. sp. (Orthoptera Tetrigidae), is described from Jagdalpur, Chhattisgarh, India. Also studied with 19 species belonging to 7 genera under two subfamilies Scelimeninae and Tetriginae under family Tetrigidae.

KEY WORDS

Orthoptera; Tetriginae; new species; Scelimeninae; Barnawapara; Durg; Kabirdham.

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INTRODUCTION

Scattered information about the occurrence of Orthoptera fauna from India had been reported by several scientists: Kirby (1914) described 5 new genera and 10 new species, besides the already known 23 genera and 73 species. Hancock (1912, 1915) studied the Indian Tetrigidae. Fletcher (1921) gave a full list of synonyms and references along with the distribution for the Tetrigidae of India and the adjacent countries. Hebard (1929) studied the South Indian Tetrigids. Günther (1935-1941) revised the subfamilies of Tetrigidae with the exception of Batrachidinae and Tetriginae. Steinmann (1964-1971a, b) compiled the taxonomical work of the family Tetrigidae in the form of Checklist to give a zoogeographical picture of the species of the world. Grant (1966) studied the genera of Batrachideinae. Shishodia (1991a) studied 'Taxonomy and zoogeography of the Tetrigidae of North eastern India' and 77 species were dealt with, of which 13 species were new, 6 were new records for India, one new genus was proposed and two new synonyms were established. Shishodia (1984, 1987 a,b, 1991a,b,c, 1993, 1995, 2000a, 2007a), Paranjape &

Bhalerao (1986), Paranjape et al. (1987), Wagan & Kevan (1992) mainly worked on the taxonomy of Tetrigoidea in India.

A little work has been done about pigmy grasshopper fauna from Chhattisgarh state i.e. Shishodia (2000b) reported 77 species of crickets and grasshoppers from Bastar, of which 13 species of Tetrigidae family. Recently Skejo & Gupta (2015) gave a specific status of *Hedotettix cristatus* Karny, 1915. Gupta (2016 a, b) worked on Orthoptera fauna of Chhattisgarh and described two species of Family Tetrigidae: *Euparatettix dandakaranyensis* Gupta, 2016, *Ergatettix subtruncatus* Gupta, 2016; Gupta et al. (2016) described also *Hedotettix angulatus* Gupta, Shi et Chandra, 2016.

The genus *Coptotettix* Bolivar, 1887 is the most diverse genus of Tetrigidae erected by Bolivar (1887). This genus includes 79 species and subspecies (Eades et al., 2017), of which, 12 species reported from India (Shishodia et al., 2010).

During the extensive and intensive survey of these areas from 2011 to 2014, a total of 20 species belonging to 7 genera under 2 subfamilies Scelimeninae and Tetriginae under family Tetrigidae with a new species were collected from different

localities from Chhattisgarh and the results are shown below.

MATERIAL AND METHODS

The survey was carried out in Chhattisgarh state, covering four districts Durg, Jagdalpur, Korba and Surgaja and seven Protected areas i.e. Sitanadi Wildlife Sanctuary, Udanti Wildlife Sanctuary, Achanakmar Wildlife Sanctuary, Gurughasidas National Park, Badalkhol Wildlife Sanctuary, Barnawapara Wildlife Sanctuary and Boramdev Wildlife Sanctuary.

The specimens were collected by sweeping over pebbles on the banks of rivers, by using an insect net and killed by benzene vapor in killing jar. The specimens were preserved dry and pinned and they were studied under a Leica stereozoom Microscope (Leica M205 A) and photographs were taken using the software Leica Application Suite (LAS V3.8). All the measurements are given in millimetres. The type specimens were deposited in the Central Entomological Laboratory (CEL) of the Zoological Survey of India in Kolkata while the other specimens in the National Zoological Collection of the Zoological Survey of India, Kolkata.

All the specimens was collected by S.K. Gupta and colleagues.

ABBREVIATIONS. DC: Day collection; FRH: Forest Rest House; NC: Night collection; NP: National Park; WLS: Wildlife Sanctuary.

RESULTS

Systematics

Order ORTHOPTERA Olivier, 1789
Suborder CAELIFERA Ander, 1939
Superfamily TETRIGOIDEA Rambur, 1838
Family TETRIGIDAE Rambur, 1838
Subfamily SCALIMENINAE Rambur, 1838
Genus *Thoradonta* Hancock, 1909

1. *Thoradonta apiculata* Hancock, 1915

Thoradonta apiculata - Hancock, 1915: 8
Thoradonta apiculata - Shishodia, 2000a: 233

EXAMINED MATERIAL. Chhattisgarh, Dhamtari, Sitanadi WLS, Sankra FRH, 01.VII.2012, 1 female, NC; Durg, Badbhum FRH, 28.III.2014, 1 male, NC; Bilaspur, Achanakmar WLS, Chhaparwa FRH, 10.VI. 2012, 1 female, NC.

DISTRIBUTION. India: Andaman & Nicobar Islands, Arunachal Pradesh, Assam, Chhattisgarh, Manipur, Meghalaya, Orissa, Tamil Nadu and West Bengal. Elsewhere: Myanmar.

2. *Thoradonta spiculoba* Hancock, 1912

Thoradonta spiculoba - Hancock, 1912: 138
Thoradonta pruthii - Shishodia, 2000b: 27-80.

EXAMINED MATERIAL. Chhattisgarh, Korba, Telimnala, 03.VI.2012, 1 male, DC, Jashpur, Badalkhol WLS; Bachhram beat, 25.V.2012, 9 females, DC; Dhamtari, Sitanadi WLS; Gidhava, 21.X.2012, 1 female, DC; Koriya, Gurughasidas NP, Sonhut FRH, 19.III.2014, 1 male, NC; Durg, Badbhum FRH, 28.III.2014, 1 male, DC; Surguja, Taravayaya Nala, 17.IX.2012, 1 female.

DISTRIBUTION. India: Chhattisgarh, Karnataka, Madhya Pradesh, Maharashtra, Orissa and West Bengal.

3. *Thoradonta nodulosa* (Stål, 1861)

Tetrix nodulosa - Stål, 1861: 346
Thoradonta nodulosa - Shishodia, 2000a: 233

EXAMINED MATERIAL. Chhattisgarh, Jashpur, Badalkhol WLS; Bachhram beat, 25.V.2012, 1 male, DC; Koriya, Gurughasidas NP, Sonhut FRH, 19.III.2014, 1 male, NC.

DISTRIBUTION. India: Andaman & Nicobar Islands, Arunachal Pradesh, Assam, Bihar, Chhattisgarh, Himachal Pradesh, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Sikkim, Tamil Nadu, Tripura and West Bengal. Elsewhere : Borneo, China, Hainan, Java, Malaysia, Myanmar, Perak, Pinang, Singapore, Sri Lanka, Sumba and Sumatra.

4. *Thoradonta bengalensis* Shishodia, 1991

Thoradonta bengalensis - Shishodia, 1991a: 34
Thoradonta bengalensis - Shishodia, 2003: 82.

EXAMINED MATERIAL. Chhattisgarh, Raipur, Barnawapara WLS; Gabod, 08.X.2011, 1 male, DC; Parbatiya Nala, 04.VI.2012, 1 male, NC; Domar pani, 24.IV.2012, 1 female, DC; Jagdalpur, Sonarpal beat, 17.X.2011, 3 males, DC; Dhamtari, Sitanadi WLS; Sitanadi river, 1 female, DC, 17.X.2011, 1 female, DC; Kabirdham, Bhoramdev WLS; Rajadhar Forest, 01.XII.2011, 1 female, DC; Jashpur, Badalkhol WLS; Bachhram beat, 25.V.2012, 11 females, DC; Korba, Kudmura nala, 31.V.2012, 1 male, DC; Bilaspur, Achanakmar WLS, Karidongri FRH, 19.IX.2012, 1 female, DC; Gariyaband, Udanti WLS; Kulhadighat, 28.VI.2014, 1 male, DC.

DISTRIBUTION. India: Chhattisgarh and West Bengal.

Genus *Euscelimena* Günther, 1938

5. *Euscelimena harpago* (Serville, 1838)

Tetrix harpago - Serville, 1838: 763

Scelimena uncinata - Shishodia, 1991b: 34

Euscelimena harpago - Kulkarni & Shishodia, 2005: 326.

EXAMINED MATERIAL. Chhattisgarh, Raigarh, Gomerda WLS; Kanakabira, 19.II.2012, 2 males, 3 females, DC; Gariyaband, Udanti WLS; Jhariya bahara, 24.VI.2014, 3 males, 5 females, DC; 29.VI.2014, 1 female, DC.

DISTRIBUTION. India: Andhra Pradesh, Bihar, Karnataka, Gujarat, Madhya Pradesh, Maharashtra, Orissa, Tamil Nadu and Uttar Pradesh. Elsewhere: Sri Lanka.

Subfamily TETRIGINAE Serville, 1838

Genus *Paratettix* Bolivar, 1887

6. *Paratettix histricus* (Stål, 1861)

Tetrix histricus - Stål, 1861: 347

Euparatettix histricus - Shishodia, 1987b: 93

Paratettix histricus - Storozhenko & Dawwrueng, 2015: 530.

EXAMINED MATERIAL. Durg, Durg camp, 23.VIII.2011, 1 male, DC; Rampur, 24.VIII.2011,

6 females, DC; Raipur, Barnawapara WLS; Domarpani, 28.VI.2012, 1 female, DC; Nawapara, 19.VII.2013, 1 female, DC; Dhamtari, Sitanadi WLS; Gidhava, 21.X.2012, 2 females, DC; Gahnasiyar, 20.VIII.2013, 1 male, DC.

DISTRIBUTION. India: Andaman and Nicobar Islands, Andhra Pradesh, Arunachal Pradesh, Assam, Bihar, Chhattisgarh, Delhi, Himachal Pradesh, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Orissa, Rajasthan, Tamil Nadu, Tripura and West Bengal. Elsewhere: Australia, Borneo, Caledonia, Celebes, East Africa, East Afghanistan, Holland, Indonesia, Iran, Java, Malaysia, Myanmar, Nepal, New Ireland, Pakistan, Philippines, Queensland, Saudi Arabia, Solomon Islands, South China, Sumatra, Sri Lanka and Taiwan.

7. *Paratettix cingalensis* (Walker, 1871)

Tettix cingalensis - Walker, 1871: 827

Paratettix variegatus - Bolivar, 1887: 280

Paratettix cingalensis - Chandra et al., 2007: 2686

EXAMINED MATERIAL. Chhattisgarh, Raipur, Barnawapara WLS; Barnawapara, 04.IX.2011, 1 female, NC; 06.VII.2011, 1 male, NC.

DISTRIBUTION. India: Arunachal Pradesh, Assam, Chhattisgarh, Goa, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Meghalaya, Manipur, Orissa, Sikkim, Tamil Nadu and Tripura. Elsewhere: Borneo, Hainan, Malaysia, Java, Philippines, Sri Lanka, Sumatra and Taiwan.

Genus *Euparatettix* Hancock, 1904

8. *Euparatettix nodulosus* Hancock, 1912

Euparatettix nodulosus - Hancock, 1912: 154

Ergatettix nodulosus - Hebard, 1929: 587

Ergatettix guentheri - Steinmann, 1970a: 234

Euparatettix nodulosus - Gupta, 2016a: 121

EXAMINED MATERIAL. Chhattisgarh, Koriya Gurughasidas NP; 28.IX.2011, 1 male, DC; Uravpara, 24.X.2011, 1 male, DC; Raipur, Barnawapara WLS; Gabod, 08.X.2011, 1 male, DC; Latadadar, 01.VII.2013, 1 male, DC; Durg, Badbhum FRH, 28.III.2014, 1 male, 1 female, NC; Saldevi mandir, 25.IX.2012, 1 male, DC.

DISTRIBUTION. India: Arunachal Pradesh, Assam, Bihar, Chhattisgarh, Himachal Pradesh, Madhya Pradesh, Maharashtra, Meghalaya, Mizoram, Orissa, Tripura, Uttar Pradesh and West Bengal. Elsewhere: Bangladesh, Nepal and Sri Lanka.

Genus *Ergatettix* Kirby, 1914

9. *Ergatettix callosus* (Hancock, 1915)

Indatettix callosus - Hancock, 1915: 130

Indatettix callosus - Fletcher, 1921: 35

Ergatettix callosus - Shishodia, 1993: 205

EXAMINED MATERIAL. Chhattisgarh, Jagdalpur, Bhanpuri Forest, 20.X.2011, 1 male, DC; Ulnar village, 16.III.2012, 1 male, DC; Raipur, Barnawapara WLS; Hardi Nala, 29.VI.2013, 1 male, DC; Lalbandh Pond, 13.VII.2013, 1 male, DC.

DISTRIBUTION. India: Chhattisgarh and West Bengal.

10. *Ergatettix interruptus* (Brunner von Wattenwyl, 1893)

Paratettix interruptus - Brunner von Wattenwyl, 1893: 104

Euparatettix interruptus - Kirby, 1910: 31

Indatettix interruptus - Hancock, 1915: 130

Ergatettix interruptus - Blackith, 1992: 54

EXAMINED MATERIAL. Chhattisgarh, Raipur, Barnawapara WLS; Bar forest, 29.V.2013, 1 male, DC; Dhamtari, Sitanadi WLS; Bhainsa sankra, 30.VIII.2013, 1 male, DC.

DISTRIBUTION. India: Chhattisgarh. Elsewhere: Laos, Myanmar and Thailand.

11. *Ergatettix dorsifera* (Walker, 1871)

Tettix dorsifera - Walker, 1871: 825

Ergatettix dorsiferus - Shishodia, 2000: 21

EXAMINED MATERIAL. Chhattisgarh, Raipur, Barnawapara WLS; Gabod, 08.X.2011, 1 male, DC; Hardi Pathar, 29.III.2012, 1 male, DC; 30.III.2012, 1 male, DC; Keduva Nala, 03.VI.2013, 1 male, DC; Hardi Nala, 26.VI.2013, 1 male, DC;

Latadadar, 28.VI.2013, 1 male, DC; Durg; Rampur, 24.VIII.2011, 1 female, DC; Jagdalpur, Magrapara, 06.I.2012, 1 female, DC; Belguda, 18.I.2012, 1 female, DC; Malgaon, 09.III.2012, 1 female, DC; Surguja, Tara Rest House, 16.IX.2012, 1 male, DC; Koriya; Gurughasidas NP; Uravpara, 24.X.2011, 1 male, DC.

DISTRIBUTION. India: Andaman & Nicobar Islands, Arunachal Pradesh, Assam, Bihar, Chhattisgarh, Delhi, Himachal Pradesh, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Orissa, Rajasthan, Sikkim, Tripura, Tamil Nadu, Uttarakhand, Uttar Pradesh and West Bengal. Elsewhere: Afghanistan, Bangladesh, China, Central Asia, Java, Greater Sunda Islands, Iran, Indonesia, Myanmar, Nepal, Sri Lanka, Sumatra, Taiwan and Turkmenistan.

Genus *Coptotettix* Bolivar, 1887

12. *Coptotettix tricarinatus* Shishodia, 1991

Coptotettix tricarinatus - Shishodia, 1991a: 169

Coptotettix tricarinatus - Shishodia, 2003: 83

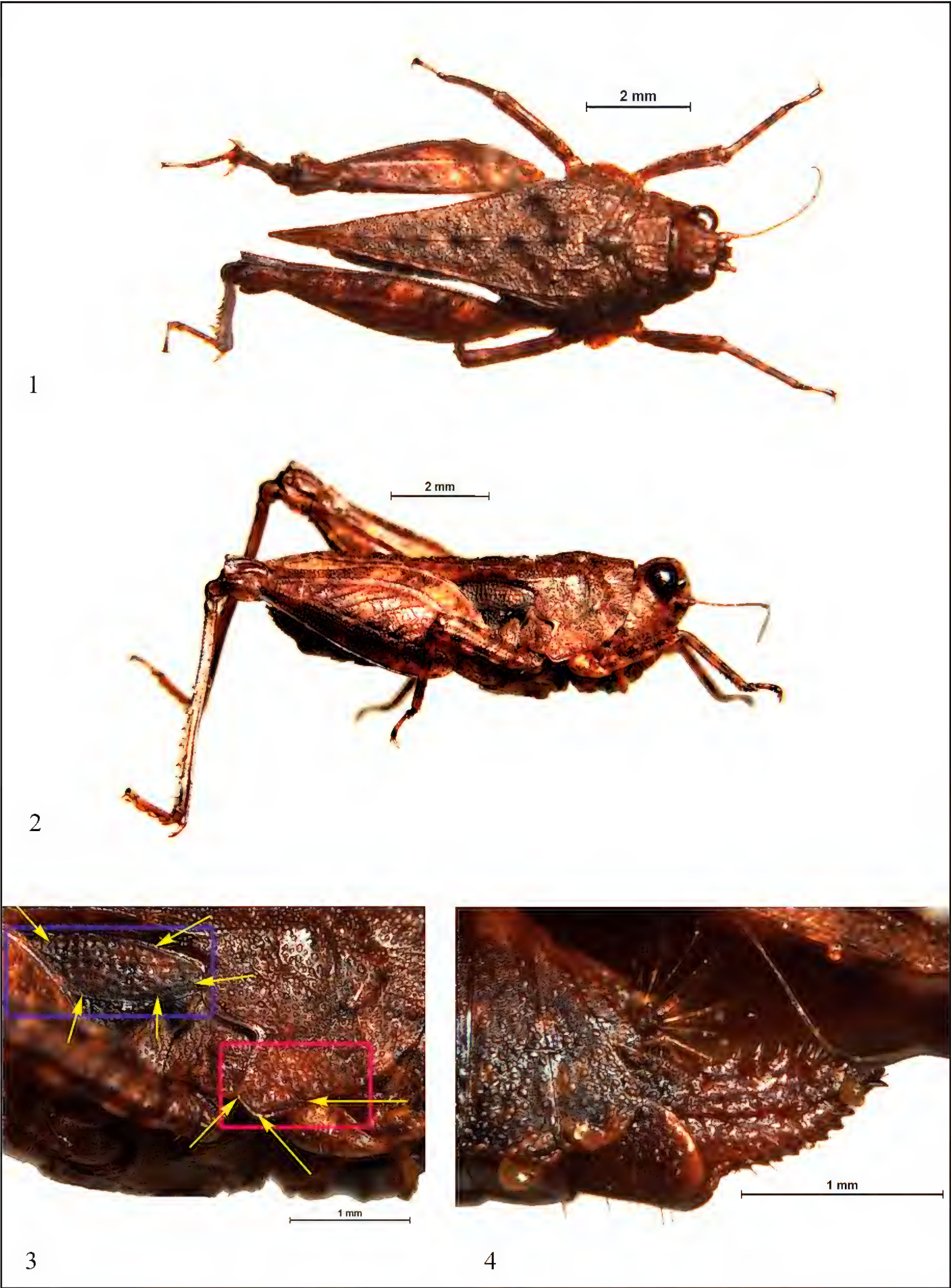
EXAMINED MATERIAL. Chhattisgarh, Jagdalpur, Malgaon, 09.III.2012, 1 male, DC; Koriya Gurughasidas NP; Sonhut FRH, 05.V.2013, 1 male, NC.

DISTRIBUTION. India: Chhattisgarh and West Bengal.

13. *Coptotettix magedhensis* n. sp. (Figs. 1–4)

EXAMINED MATERIAL. Holotype, female, DC. India, Chhattisgarh, Jagdalpur, Makdi, Magedha, 09.XI.2011, 19°05'45.6"N, 82°03'9.3"E, 592 m, legit S.K. Gupta and colleagues, Registration no. 18713/H5. Paratypes: 1 female, same data as holotype. Registration no. 187714/H5. 1 female, Jagdalpur, Hathguda, 29.III.2012, 19°05'45.6"N, 82°03'9.3"E, 561 m. Registration no. 187715/H5.

DESCRIPTION OF HOLOTYPE. Female. Vertex narrower than a compound eye, little elevated, median carina of the vertex present in the distal half of the length of vertex, elevated, fossulae present. Lateral carinae of the vertex present, strongly elevated, with tubercles. Median ocellus situated below the level of the lower margin of the compound eyes, in the place where facial carina ends. Lateral ocelli situ-



Figures 1–4. *Coptotettix magedhensis* n. sp., holotype female. Fig. 1: dorsal view. Fig. 2: lateral view. Fig. 3: Paranota and Tegmen. Fig. 4: Ovipositor.

ated in the level of the distal margin of the compound eyes. Frontal costa bifurcation starts in about upper $\frac{1}{4}$ of the compound eye height and runs parallel to the median ocelli forming a wider scutellum. Antennal grooves are situated at the level of the lower margin of compound eyes or slightly below it. Eyes in frontal view sub-globular, in lateral view sub-globular. Eyes can be not close to the pronotum so the occipital area is visible.

Pronotum. Pronotum granulated with fine, medium sized and large granules, wrinkled. Anterior margin truncate, extended upto the last abdominal segment, acute at apex. Median carina is continuous towards the whole length of pronotum bearing 6–7 small projections of variable height and size. External carinae fused to humero-apical carinae. Humero-apical carinae forming with external lateral carinae obtuse angle, two lateral lobes present. Posterior margin of lateral lobe truncated.

Wings. Tegmina elongated, wings present, visible under pronotum extend up to 4th or 5th abdominal segments.

Fore legs: femora 3 times as long as wide, Upper and lower margins of femora straight; serration on external face indistinct; tibia clothed with 7 spines and some small hairs; tibial serration on external face distinct, second tarsal segments 5 times longer than first.

Mid legs: femora 3.6 times as long as wide, serrated carina on external face distinct, Upper and lower margins of femora slightly undulate, upper and lower mid carinae with several black warts, tibiae clothed with 4 spines and some hairs; second tarsal segments 2.9 times longer than first.

Hind legs: Hind femora robust 2.6 times as long as wide, upper margins serrated, tibiae with 8 outer and 5 inner spines. first tarsal segments 1.5 times longer than third; tip of pulvilli I and II spinulately produced and pulvilli III obtusely, pulvilli II distinctly longer than length of I and also II pulvilli. Subgenital plate in ventral view square shaped; Ovipositor elongated. Epiproct as long as wide near the base, with obtuse apex. Cerci cylindrical, wide near the base with obtuse tip.

Coloration. Body color from brown to dark blackish. Pronotal tubercles usually dark black. Fore tibiae, mid tibiae and hind tibiae with two dark brown hues, genicular lobe of hind femora dark brown; fore legs of III tarsal segment with dark

black brown hue. Ventral region of hind femora dark black color. Tip of spines dark blackish.

Measurements (all in millimeters). Body length Female. (from the tip of the vertex to the end of the abdomen): 8.959; head length: 0.944; Vertex: 0.582. compound eye length: 0.659, width: 0.391. Antenna length: 2.994. Antenna groove length: 0.145, width: 0.223, scapes length: 0.219, pedicel length: 0.177, scutellum width: 0.212. Pronotum length: 7.691, shoulder width: 2.383. Fore leg: femur length: 1.535, width: 0.501; tibia length: 1.760, width: 0.214; tarsus length (by segments): I: 0.114, II: 0.577; claw length: 0.192. Mid leg: femur length 1.776, width 0.482; tibial length 1.692, width 0.262; tarsus length: I: 0.184, II: 0.538; claw length 0.287. Hind leg: femur length: 4.856, width: 1.807; tibia length: 3.808, width: 0.236; tarsus length: I: 0.712, II: 0.132, III: 0.465; pulvilli length: I: 0.245, II: 0.226, III: 0.312; claw length 0.226. Abdomen length: 4.886. Wings: tegmen length: 1.393, width: 0.450; Dorsal ovipositor valves length: 1.252, height: 0.302; ventral ovipositor valves length: 1.048, height: 0.149; cerci length: 0.310, width: 0.125.

VARIABILITY. The paratypes female have no substantial morphological differences from the holotype described. Male unknown.

ETYMOLOGY. The specific epithet refers to the type locality.

DISTRIBUTION. *Coptotettix magedhensis* n. sp. is known actually from the type locality only: India, Chhattisgarh.

Genus *Criotettix* Bolivar, 1887

14. *Criotettix bispinosus* (Dalman, 1818)

Acrydium bispinosum - Dalman, 1818: 77

Criotettix bispinosus - Günther, 1937: 123

Criotettix bispinosus - Shishodia, 2000a: 232

EXAMINED MATERIAL. Chhattisgarh, Koriya, Gurughasidas NP, Ghatoriya Pahad, 28.XI.2013, 1 male, DC; Thitima Pahar, 24.X.2013, 1 male, 1 female, DC.

DISTRIBUTION. India: Arunachal Pradesh, Assam, Bihar, Chhattisgarh, Madhya Pradesh, Manipur, Meghalaya, Tripura and West Bengal. Else-

where: Borneo, Celebes, China, Hainan, Hong kong, Java, Luzon, Malaysia, Myanmar, Sulawesi, Sumatra, Taiwan, Thailand and Vietnam.

Genus *Hedotettix* Bolivar, 1887

15. *Hedotettix attenuatus* Hancock, 1904

Hedotettix attenuatus - Hancock, 1904: 151

Hedotettix attenuates - Kirby, 1914: 73

Hedotettix attenuatus - Chandra et al., 2007: 2687

EXAMINED MATERIAL. Chhattisgarh, Raipur, Barnawapara WLS; Barnawapara, 24.VII.2011, 1 male, 1female, NC; Raigarh, Gomerda WLS; Naresh Nagar, 24.VIII.2011, 1 male, NC; Surguja, Anjani Beat, 10.XI.2011, 1 female, DC; Korba, Paharjamri Beat, 24.XI.2011, 1 female, DC; Bijakhara, 01.VI.2012, 1 male, DC; Koriya, Gurughasidas NP; Sonhut Forest Rest House, 21.IV.2013, 1 male, 2 females, NC; 05.V.2013, 1 female, NC; 19.III.2014, 1 female, NC; Kamarjee, Bagridanar, 22.IX.2013, 1 male, DC; Thitima Pahar, 24.X.2013, 1 male, DC; Berdev Nala, 17.XI.2013, 1 male, DC; Ghatoriya Pahar, 28.XI.2013, 1 male, DC; Rewala, 09.XII.2013, 1male, 2 females, DC; Durg, Badbhum FRH, 08.II.2014, 1 female, NC; 31.III.2014, 1 female, NC; Gariyaband, Udanti WLS; Ganga Jamuna village, 28.VI.2014, 1 male, 2 females, NC.

DISTRIBUTION. India: Andaman and Nicobar Islands, Arunachal Pradesh, Assam, Chhattisgarh, Himachal Pradesh, Manipur, Meghalaya, Orissa, Sikkim, Tripura and West Bengal. Elsewhere: Sri Lanka.

16. *Hedotettix gracilis* (Haan, 1842)

Acridium gracile - Haan, 1842: 169

Hedotettix gracilis - Hancock, 1915: 123.

Hedotettix gracilis - Chandra et al., 2007: 2687

EXAMINED MATERIAL. Chhattisgarh, Raipur, Barnawapara WLS; Barnawapara, 10.VII.2011, 1 male, DC; 14.VII.2011, 2 females, NC; 20.VII.2011, 1 female, NC; 24.VII.2011, 1 female, NC; 09.XI.2011, 1 male, DC; 03.II.2012, 1 female, NC; Keduva Nala, 09.VII.2011, 1 female, DC; Dulibahal, Forest, 10.IX.2011, 1 male, DC; Latadadar,

02.I.2012, 1 female, DC; Surguja, Bengahi Pahar, 10.XI.2011, 1 male, DC; Kabirdham, Bhoramdev WLS; Sitalpani, 19.XI.2011, 1 male, DC; Sankri River, 30.IX.2013, 1 male, DC; Raigarh, Gomerda WLS; Gomerda village, 28.V.2013, 1 male, DC; Koriya, Gurughasidas NP; Rawanpat, 23.X.2013, 1 male, DC.

DISTRIBUTION. India: Andaman and Nicobar Islands, Arunachal Pradesh, Assam, Chhattisgarh, Himachal Pradesh, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Orissa, Rajasthan, Sikkim, Tripura, Uttarakhand, Uttar Pradesh and West Bengal. Elsewhere: Bangladesh, Celebes, China, Java, Myanmar, Pakistan, Sri Lanka, Sulawesi, Sumatra, Taiwan, Thailand and Vietnam.

17. *Hedotettix costatus* Hancock, 1912

Hedotettix costatus - Hancock, 1912: 147

Hedotettix costatus - Shishodia & Tandon, 2000: 221

EXAMINED MATERIAL. Chhattisgarh, Raipur, Barnawapara WLS; Latadadar, 27.IX.2011, 1 female, NC.

DISTRIBUTION. India: Assam, Bihar, Himachal Pradesh, Meghalaya, Sikkim, Tripura, Uttar Pradesh and West Bengal. Elsewhere: Bangladesh, Nepal and Sulawesi.

18. *Hedotettix punctatus* Hancock, 1909

Hedotettix punctatus - Hancock, 1909: 422

Hedotettix punctatus - Shishodia, 2007: 89

EXAMINED MATERIAL. Chhattisgarh, Raipur, Barnawapara WLS; Gabod, 08.X.2011, 1 male, DC; Koriya; Gurughasidas NP; Dev Nala, 09.III.2013, 1 male, DC; Durg, Badbhum FRH, 30.III.2014, 1 male, DC; Kabirdham, Bhoramdev WLS; Sankri River, 30.IX.2013, 2 females, DC); Gariyaband, Udanti WLS; Jhariyabahara, 22.II.2014, 1 male, DC.

DISTRIBUTION. India: Chhattisgarh, Karnataka, Madhya Pradesh, Maharashtra and Rajasthan. Elsewhere: Borneo and Himalayan region.

19. *Hedotettix grossus* Hancock, 1915

Hedotettix grossus - Hancock, 1915: 124

Hedotettix grossus - Shishodia, 2000: 243

EXAMINED MATERIAL. Chhattisgarh, Raipur, Barnawapara WLS; Nawapara, 23.VII.2011, 1 female, NC; Kabirdham, Bhoramdev WLS; Kherpani Forest, 16.XI.2011, 1 female, NC; Sankri river, 30.IX.2013, 1 female, NC; Dhamtari, Sitanadi WLS; Bhainsa Sankra, 04.IX.2013, 1 female, DC; Sankra FRH, 24.II.2014, 1 female, NC.

DISTRIBUTION. India: Arunachal Pradesh, Assam, Chhattisgarh, Manipur, Meghalaya, Tripura and West Bengal.

20. *Hedotettix quadriplagiatus* (Walker, 1871)

Tettix quadriplagiatus - Walker, 1871: 828

Hedotettix quadriplagiatus - Blackith, 1992: 88

EXAMINED MATERIAL. Chhattisgarh, Kabirdham, Bhoramdev WLS; Sitalpani, 19.XI.2011, 1 male, DC; Rajadhar Forest, 01.XII.2011, 1 female, DC; Gariyaband, Udanti WLS; Jhariyabahara, 24.IV.2014, 1 male, 3 females, DC; Durg, Badbhum FRH, 31.III.2014, 2 females, DC.

DISTRIBUTION. India: Chhattisgarh and North India.

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Further lion, *Panthera leo senegalensis* Meyer, 1826, sightings in Mole National Park, Ghana, and possible first serval *Leptailurus serval* Schreber, 1776 record after 39 years (Mammalia Felidae)

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ABSTRACT

In this note we report on two recent sightings of lion, *Panthera leo senegalensis* Meyer, 1826 (Mammalia Felidae), one of which made by the authors, in Mole National Park (Ghana) during our last field research in April, 2015. We also obtained a camera trap picture of a probable serval individual, *Leptailurus serval* Schreber, 1776 (Mammalia Felidae), a species not recorded in the Park since 1976. Our conclusions indicate how the cat status in Mole National Park is very little known.

KEY WORDS

lion; *Panthera leo*; serval; *Leptailurus serval*; Mole National Park; Ghana.

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INTRODUCTION

The western African lion population, *Panthera leo senegalensis* Meyer, 1826 (Mammalia Felidae), is Critically Endangered (Henschel et al., 2014) and currently occupies only 1% of its historical range. Currently the presence of this subspecies is confirmed only in some protected areas of Senegal, Burkina Faso, Benin, Niger and Nigeria (Henschel et al., 2014).

A particularly interesting area, due to its strategic position between the above mentioned surviving populations, is the Mole National Park (MNP), Northern Region, Ghana. Here the species has always been present in historical times (Grubb et al., 1998; Angelici et al., 2015), but the last empirical evidence is an adult male shot in 2004 (Burton et al., 2010). Currently, lion is considered functionally extinct or extirpated in the whole Ghana (Henschel

et al., 2010, 2014). Despite this, indirect evidence which seems to indicate the persistence of a small population has been found and published in literature (Angelici & Petrozzi 2010; Angelici et al., 2015).

During this research, we also obtained a camera trap picture of a probable serval individual, *Leptailurus serval* Schreber, 1776 (Mammalia Felidae), a species not recorded in the Park since 1976. The serval is Least Concern in IUCN Red List of Threatened Species (Thiel, 2015).

MATERIAL AND METHODS

From April 8th to May 5th we carried out our last field research in MNP for the project “The Pride of Ghana” (see Angelici et al., 2015). Here, we investigated a lion’s sighting occurred 18 days before

our arrival. Some day after we have been involved in a direct sighting.

The study area was the Mole National Park (MNP) in Northern Region of Ghana (Fig. 1).

RESULTS AND CONCLUSIONS

The first sighting ($09^{\circ}22.719'N$ - $001^{\circ}50.994'W$ - 128 m a.s.l., see figure 2) took place in March 22, 2015 around midday (12:00) and involved a patrol of five rangers. We personally interviewed the witnesses separately and all of them agreed in their description of an adult male in good health, observed from a distance of about 80 meters. The lion ran away in the bush after noticing the men.

Due to the distance and the extremely hard soil it was not possible for them to take pictures (the rangers don't have cameras, they only own mobile phones able to take poor quality pictures) of the animal or find clear footprints. The witnesses of the

first sighting were interviewed by us after our arrival at MNP. Despite the time elapsed since the sighting, all witnesses were in agreement and described the same details. All the rangers involved are highly experienced and with many years of service and so we believe their sighting to be reliable.

The second sighting ($09^{\circ}23.340'N$ - $001^{\circ}56.855'W$ - 165 m a.s.l., see figure 2) took place in April 17, 2015 at 8:20 p.m. and involved five persons: the authors (FMA, LR), two rangers and the driver.

While we were driving from the Mole community to Lovi for a call station session (see Ogutu & Dublin, 1998; Nyanganji et al., 2012) a lioness unexpectedly crossed the road in eastward direction, about 40-50 m from us. The sighting lasted about 15 seconds. We could clearly see the shape and the colour of the animal while it crossed the road because it was illuminated by the car headlights and by a 100 watt spotlight (Lightforce LS240) that was already at hand, because we normally used it during our night patrols by car. Un-

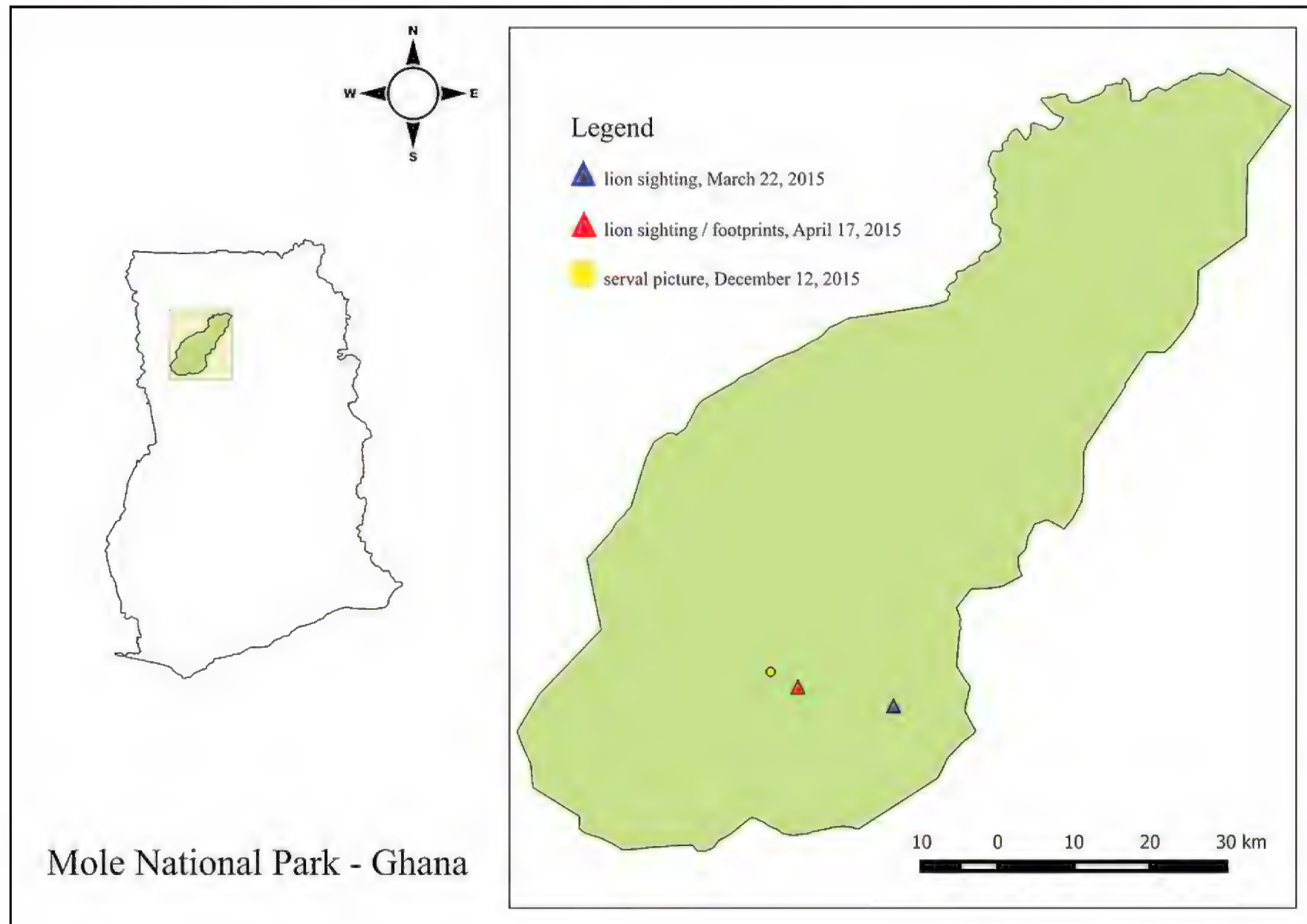


Figure 1. The study area and lion sightings recorded in Mole National Park, Ghana, during 2015.



Figure 2. Footprint of the lioness spotted on April 17, 2015.

Figure 3. probable serval picture taken on December 12, 2015.

fortunately, we had no time to extract our reflex camera from the bag and to set it to take pictures before the lioness disappeared into the bush. With the help of the headlights we found some felid footprints, discovering two distinct trails crossing the road almost perpendicularly. One trail was characterized by footprints about 10 cm long, and the second one by footprints 7.8 cm long. The trails crossed each other only in one point, and belonged to two different animals. We attributed the larger one to the lioness we saw (see Fig. 3).

The project “The Pride of Ghana” ended on 30th June 2016 and sadly we did not obtain independent verifiable evidence on the presence of lions in the Park. Despite that we believe that such investigation should continue and we invite zoologists who in future will conduct research in the Park to continue to investigate this possibility, hoping to finally get strong evidence.

According to Burton et al. (2010) some characteristics of MNP (limited access, low visibility and poor tracking substrate), make ineffective methodologies like call in station and spoor searches.

We also experienced the same issues and we believe that the best way to investigate the MNP is the use of camera traps. But even the latter doesn't avoid issues, such as theft of some camera traps by poachers and the wearing out of sensors and batteries performance due the harsh field conditions.

In date December 12th 2015, a camera trap of the project shot an intriguing, despite not perfectly

clear, picture of a felid which is not a lion nor a leopard (see Fig. 1). The other two candidate species recorded in MNP are the caracal (*Caracal caracal* Schreber 1776) and the serval (*Leptailurus serval*) and appear evident to us that the identity of the animal in the picture belongs to one of these latters.

Being caracal largely unmarked (i.e. Hunter, 2015), we believe that the most likely hypothesis is a serval. Servaline individuals, characterized by faint, speckled spotting are known mainly from West Africa (Hunter, 2015). We submitted the picture and our hypothesis to some leading cat authorities and, despite the quality and the angle of the picture can't permit a 100% accurate attribution, they agreed that the serval identity is plausible although not definitive (P. Henschel pers. comm., L. Hunter pers. comm.). Since the last serval record for the MNP dates back to 1976 (Robertson, 1977), this picture could confirm how an elusive species could be reappear even after years of gap since the last record (e.g. Ahmed et al., 2016) and could a further quest for future researches of the yet so little known biodiversity of the MNP.

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A report on two alien invasive species of the genus *Sceliphron* Klug, 1801 (Hymenoptera Sphecidae) from Sicily, with a brief faunistic update on the native species

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ABSTRACT

Two alien invasive species of the genus *Sceliphron* Klug, 1801 (Hymenoptera Sphecidae) were recently found in Sicily: *S. caementarium* (Drury, 1773) is recorded from Sicily (Messina province) for the first time; *S. curvatum* (F. Smith, 1870), previously recorded from Sicily only through generic data from literature and only one locality in a web forum of amateurs, is confirmed as definitely established in the Island. The Regional distribution of both alien species in Italy is revised based on data taken from literature and reliable reports from web forums. A brief faunistic account on the three native *Sceliphron* from Sicily is provided: *S. destillatorium* (Illiger, 1807) and *S. spirifex* (Linnaeus, 1758) are both new for the Aeolian Islands (respectively reported for Panarea and Vulcano).

KEY WORDS

Sceliphron caementarium; first record; Sphecidae; Sicily; Italy; alien; invasive species.

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INTRODUCTION

In terms of alien species diversity within invertebrate orders, Hymenoptera ranks as third following Coleoptera and Hemiptera, with about 300 species, representing 30 families, introduced to Europe (Rasplus et al., 2010). Among the Mediterranean countries, Italy hosts the largest number of alien Hymenoptera, about half of the European species, 144 according to Rasplus et al. (2010). But the knowledge of alien hymenopteran species in insular contexts still remains under-explored. It is noteworthy to underline that for islands alien species could play a more relevant role rather than mainland areas, due to obvious geographic reasons and thus the peculiar ecological context, especially in smaller islands.

The genus *Sceliphron* Klug, 1801 (Hymenoptera Sphecidae) is a distinctive sphecid-wasp genus characterized by the predominantly black colour of the

body with more or less extended yellow spots. The head has a flattened frons, antenna filiform, without placoids in the male, distance between antennal socket and fronto-clypeal suture at least 0.5 antennal socket diameter, mandible without teeth (with some exception in the female of a few species) and buccal parts short, with the third maxillary palpomere widened on its side; the legs have tarsal plantulae, without spines; the propodeum has the dorsal area sharply defined, with a longitudinal median furrow; the wings are hyaline, weakly yellowish, with both recurrent veins ending in the second submarginal cell; the metasoma has an elongate to very elongate tubular petiole (Bohart & Menke, 1976; Pagliano & Negrisola, 2005).

The *Sceliphron* species build a mud multilocular pedotrophic nest attached to a wide range of substrates, mainly rock walls, in a more or less concealed position, and frequently many specimens

work closely each other to produce massive concentration of nests (Chatenoud et al., 2012). It is not unusual to find nests in strange substrates, e.g., a wide range of handworks (Gepp, 2003; pers. obs.). The pedotrophic cells are provided with a variable number of specimens of Aranaeae.

The genus has a worldwide distribution with 35 species (Pulawski, 2017), of which eight recorded for Europe and Mediterranean basin (Schmid-Egger, 2005) and five for Italy (Pagliano & Negrisol, 2005), including both the three native and the two alien invasive species: *S. caementarium* (Drury, 1773) and *S. curvatum* (F. Smith, 1870). In Sicily three native species of the genus were recorded so far, although Strumia et al. (2012) indicated the presence of the alien *S. curvatum* since the year 2002 through a map, without indication of locality.

In the present note, we provide the first evidence of the presence in Sicily of the two alien invasive species of *Sceliphron* known for other parts of Italy.

MATERIAL AND METHODS

The examined material includes two specimens recently collected through hand-net by the authors in Messina province. The material has been identified by the first author based on the keys and diagnoses provided by Bitsch et al. (1997), Pagliano & Negrisol (2005) and Schmid-Egger (2005). To reconstruct the distribution of the treated species in Italy it has been taken into account data from specialist literature as well as reliable records (e.g., only those containing photos, thus allowing a safe identification, as well as precise collecting data) published in social forums, namely: “Forum Entomologi Italiani”, referred as F.E.I. in the text, available at <https://www.entomologiitaliani.net/forum> (accessed June 11, 2017); “Natura Mediterraneo”, section Hymenoptera, referred as Na.Me. in the text, available at <https://www.naturamediterraneo.com> (accessed June 11, 2017); “Aracnofilia, Associazione Italiana di Aracnologia”, referred in the text as A.I.A., available at <http://forum.aracnofilia.org> (accessed June 11, 2017); “Forum Acta Plantarum”, referred in the text as Ac.Pl., available at <http://www.floraitaliae.actaplantarum.org/index.php> (accessed June 16, 2017). Moreover, the status of the treated alien species has been detected in the website DAISIE (Delivering Alien Invasive Species Inventories for Europe), being the major public source for informa-

tion on biological invasions, available at <http://www.europe-aliens.org/default.do> (accessed June 17, 2017). Photographs were produced with a Canon Power Shot SX130 IS digital camera with a 12.1 mega pixels’ resolution into a light chamber with the aid of 11W fluorescent light-source.

RESULTS AND DISCUSSION

The present addition allows to raise the number of the known *Sceliphron* species of Sicily from three to five, which represent all the species currently known to Italy (Pagliano & Negrisol, 2005).

Sceliphron caementarium (Drury, 1773) (Fig. 1)

EXAMINED MATERIAL. 1 female, Sicily, Messina province, Messina, Tremestieri, 35 m a.s.l. (38°08'36.9"N/15°31'21.7"E), 23.VIII.2013, G. Altadonna leg. (G.F. Turrisi collection, Pedara, Italy).

DESCRIPTION. Main colour black, including trochanters and femurs of hind legs and petiole, except: antennal scapes, tegulae, pronotum, scutellum of mesonotum, upper part of mesopleurae, metanotum, distal part of propodeum, most of fore and mid legs (except coxae, basal half of femurs and tarsomeres 2–5), basal half of hind tibiae and hind tarsomeres 1–3, most of metasomal tergite 1 (except base), yellow; wings hyaline, sharply yellowish with distal half slightly infusate. Petiole straight, slightly shorter than rest of metasoma (ratio: about 0.9); last sternite without carina.

REMARKS. In origin, it was a Nearctic species (Pulawski, 2017), which is become very widespread in the World, being accidentally introduced into Europe, Asia, Australasia (including Fiji, Micronesia, Cook Islands), South America, Hawaiian Islands, Antille, Martinique, Marquesas Islands (Pagliano et al., 2000a, 2000b; Pagliano & Negrisol, 2005; Schmid-Egger, 2005; Pulawski, 2017). In Europe, it has been introduced several times during 19th and 20th centuries (Rasplus, 2010); it is believed that the introducing way was mainly by ship cargos (Berland, 1946; Bogusch & Macek, 2005). It was first reported in 1945 from Versailles (but was never subsequently reported there) and in 1949 from southern France (Berland, 1946; Rasplus, 2010). Bogusch & Macek (2005) documented the possible first finding of the species in Europe, through a spe-

cimen collected in 1942. Anyway, only since the 1970s this species becomes well established in many countries of Europe and Asia: Belgium, Luxembourg, France (including Corsica), Germany, Austria, Switzerland, Czech Republic, Slovakia, Croatia, Italy, Spain (including Canary Islands), Portugal, Ukraine, Iran, India (Leclercq, 1974, 1975; Haeseler, 1976; Erlandsson, 1978; Leclercq & Claparede, 1978; Schneider & Pells, 1988; Hamon et al., 1989; Pauly, 1999; Pagliano, 1992, 1995; Pagliano et al., 2000a, b; Gusenleitner, 2002; Vernier, 2003; Hellrigl, 2004; Bogusch & Macek, 2005; Pagliano & Negrisol, 2005; Schmid-Egger, 2005; Bitsch & Barbier, 2006; Veprek & Straka, 2007; Fallahzadeh et al., 2009; Pagliano, 2009; Bitsch, 2010, 2014; Rasplus, 2010; Callot & Brua, 2013; Kannagi et al., 2013; Zettel et al., 2014; Dollfuss, 2016; DAISIE, 2017). However, the distribution at country level still remains under-explored and in need of further investigation above all to establish whether its presence also includes natural habitats and which types. In Italy, *S. caementarium* was found for the first time in 1990 in Tuscany, near Pisa (Strumia, 1996). Field observations evidence an apparent slow rate of spread, about 30 km in eight years (Pagliano et al., 2000a, 2000b). Based on literature, the presence of the species in Italy covers the following Regions: Liguria, Piedmont, Veneto, Valle d'Aosta, Lombardy, Trentino-Alto Adige, Friuli-Venezia Giulia, Emilia-Romagna, Tuscany (including Elba and Montecristo), Marche, Latium, Umbria, Abruzzo, Molise, Campania, Apulia, Basilicata, Sardinia (Pagliano, 1992, 1995; Strumia, 1996; Campadelli et al., 1999; Hellrigl, 2004; Pagliano & Negrisol, 2005; Pavesi, 2008; Pagliano, 2009; Ceccolini & Paggetti, 2011, 2012a, 2012b; Strumia et al., 2012; Cillo & Bazzato, 2013; Dollfuss, 2016; F.E.I., 2017; Na.Me., 2017; A.I.A., 2017; Ac.Pl., 2017). The species is newly recorded from Sicily, becoming the southernmost Italian area with its presence.

The biology of the species has been largely studied in both native (e.g. Eberhard, 1970; Powell & Taylor, 2017) and non-native areas (e.g. Chatterjee, 2015). In Europe, some biological aspects have been studied in some detail by Campadelli et al. (1999), with data on nest, preys, phenology, parasitoids and inquilines. The collecting site is comprised in the wide urban context of Messina.

Sceliphron curvatum (F. Smith, 1870) (Fig. 2)

EXAMINED MATERIAL. 1 female, Sicily, Messina

province, Caronia, Torre del Lauro, 5 m a.s.l. (38°02'39.75"N/14°31'57.44"E), 30.VI.2014, G.F. Turrisi leg. (G.F. Turrisi collection, Pedara, Italy).

DESCRIPTION. Main colour black, including trochanters and femurs of hind legs and petiole, except: clypeus, ventral part of antennal scapes, apex of antennomeres 3-4, tegulae, pronotum, middle part of mesoscutellum, upper part of mesopleurae, two spots on base and distal part of propodeum, ventral surface of distal half of fore and mid legs, distal half of metasomal tergite 1, yellow; tibiae, basal half of hind femurs and tarsi yellowish orange; metasomal tergites other than 2, with a discontinuous pale yellowish band; wings hyaline, only slightly yellowish along a narrow line of costal to marginal cell. The yellow colour could vary from yellow to yellowish-brown and reddish orange (e.g., see photos at F.E.I., Na.Me., and Ac.Pl.). Petiole sharply arcuate upwards, quite shorter than rest of metasoma (ratio: about 0.5); last sternite with a weak but distinct carina carinate.

REMARKS. According to Hensen (1987), originally the species seemed to be confined to mountainous regions South and West of the Himalayas, in Tajikistan, Pakistan, India and Nepal. Currently, its distribution covers a wider area including Iraq, Iran, Turkmenistan, Uzbekistan, Kyrgyzstan, Afghanistan, with extension to Central and South Europe and recently recorded also from South America: Argentina and Chile (Hensen, 1987; Schmid-Egger, 2005; Compagnucci & Roig Alsina, 2008; Barrera-Medina & Garcete-Barrett, 2008; Četković et al., 2011; Kannagi et al., 2013; Gulmez & Can, 2015; Dollfuss, 2016; Yildirim et al., 2016; Pulawski, 2017). In Europe, it was first recorded in southeastern Austria apparently being probably accidentally introduced in the 1970s, with a specimen collected in 1979 (van der Vecht, 1984). Later, Gepp (1995) added new records from Austria with a detailed reconstruction of the possible "first road" of the species in Europe, through a north-eastern direction from the first documented recorded locality: Steiermark, 40 km south-western of Graz, to Wien during the years 1979–1992. The distribution of the species in Europe, recently revised by Četković et al., 2011 (with many additional updates, see below), appears to be wide, with a remarkable expansion of the range in all directions. It is believed that the species in Europe has spread on its own through the main river valleys, as suggested by Schmid-Egger (2005) and

thus, the accidental dispersal by human activities was of less importance. Based on the extensive available literature, the present European and Middle Asian distribution of the species covers the following Countries: The Netherlands, Germany, Poland, Austria, Czech Republic, Hungary, Bulgaria, Slovenia, Croatia, Serbia, Montenegro, Spain (including Balearic Islands), Portugal, France (including Corsica), Italy (including Sardinia and Sicily), Caucasus, Ukraine, Georgia, Turkey, Kyrgyzstan, Tajikistan, Uzbekistan (Van der Vecht, 1984; Scaramozzino, 1995, 1996; Gogala, 1995; Gusenleitner, 1996; Pagliano et al., 2000a, 2000b; Gonseth et al., 2001; Osten, 2003; Shorenko, 2003; Četković et al., 2004, 2011; Bogusch et al., 2005; Jacobi, 2005; Schmid-Egger, 2005; Bitsch & Barbier, 2006; Gayubo & Izquierdo, 2006; Vepřek & Straka, 2007; Castro, 2007; 2010; Bury et al., 2009; Guéorgiev & Ljubomirov, 2009; Lopez-Villalta, 2009; Bitsch, 2010, 2014; Dobosz, 2010; Prokofiev & Skomorokhov, 2010; Imre, 2012; Schardt et al., 2012; Callot & Brua, 2013; Olszewski et al., 2013; Rey-Muniz, 2013; Wiśniowski et al., 2013; Bilanski et al., 2014; Khvir, 2014; Schneider et al., 2014; Gulmez & Can, 2015; Dollfuss, 2016; Yildirim et al., 2016; DAISIE, 2017). However, the distribution at country level still remains under-explored and in need of further investigation to establish whether its presence also includes natural habitats and which types.

In Italy, it is documented as established since the year 1995 in Piedmont (Torino), Friuli-Venezia Giulia (Lignano Sabbiadoro, Udine province) and Emilia-Romagna (Bologna) (Scaramozzino, 1995, 1996; Pagliano et al., 2000a, 2000b; Pagliano & Negrisol, 2005; Pagliano, 2009). Further subsequent observations clearly indicate that this species is become widely present in the whole Italy including Islands: Liguria, Piedmont, Lombardy, Veneto, Friuli-Venezia Giulia, Trentino Alto-Adige, Emilia-Romagna, Tuscany, Latium, Marche, Molise, Abruzzo, Campania, Basilicata, Calabria, Sardinia and Sicily (Scaramozzino, 1995, 1996; Grillenzoni & Pesarini, 1998; Pagliano et al., 2000a, b; Hellrigl, 2001, 2002, 2004, 2005; Jacobi, 2005; Cillo et al., 2009; Pagliano, 2009; Olivieri, 2010; Adamo, 2011; Ceccolini & Paggetti, 2011, 2012c; Chatenoud et al., 2012; Strumia et al., 2012; F.E.I., 2017; Na.Me., 2017; Ac.Pl., 2017). The available data seem to indicate that the spread of *S. curvatum* in Italy started in the mid 1990s in the Northern and quickly expanded its range in the Mid and Southern areas to

reach Calabria and Sicily presumably in the year 2002 as indicated by Strumia et al. (2012). The species, in the literature, has only been roughly indicated as present in Sicily through a map (Rasplus, 2010; website DAISIE, 2017, see Material and Methods). Strumia et al. (2012) indicated an unknown locality of Messina province through in a map. The unique precise record for Sicily is that of Cammarata (Agrigento province) by Piazza S., with a photographic report showing nest and adult observed on April 07, 2013 (Na.Me., available at https://www.naturamediterraneo.com/forum/topic.asp?TOPIC_ID=201489). The new record confirms the presence of the species in the north-eastern Sicily. The several records allow to indicate this alien species as definitely established in Sicily, where it is apparently widespread.

The biology of the species in Europe has been studied in some detail by Gepp (1995) with data on nest, preys, phenology, morphology of larva, pupa and images; further interesting data on host range were added by Carrière (2005), Dorow & Jäger (2005), Rahola (2005) and more recently by Četković et al. (2011), Csaba & Imre (2015), Gulmez & Can (2015), Madl & Vidlar (2005), and Schedl (2016).

The studied specimen has been collected in a semi-natural area close to the coast, outside the small village Torre del Lauro, in Caronia municipality, the nearest urban center being Acquedolci, about 5 km eastwards. It was not possible to locate the nesting site of the species; the specimen was observed to collect mud in a small creek.

OBSERVATIONS ON INVASIVENESS OF THE ALIEN *SCELIPHRON*

Sphecids wasps of the genus *Sceliphron* are good and active colonizers, they are generalist spider predators, they have good features to live and nest in human settlements and have a good kind of dispersion. As far as it is known, among the two-alien species, *S. caementarium* seems to be more invasive than *S. curvatum*, since the latter shows a narrower habitat preferences rather than the other three native species. The possible ecological impact of the allochthon *S. curvatum* over the native *Sceliphron* species has been discussed by Gepp (2003), especially referring to the native *S. destillatorium*, due to the apparent decreasing frequency of observations of the native species. Anyway, the invas-

iveness and thus the impact of *S. curvatum* on native *Sceliphron* species is still poorly understood and mostly anecdotic as underlined by Lukas et al. (2006), and needs further study in a wider geographic and ecological scale. Based on available data, this alien species seems to be more synanthropic than the other native species, e.g., *S. destillatorium*, showing preference for urban areas and nesting at human buildings and dry and hot places (Schedl, 2016). This narrower ecological niche in Europe should predict that the possible competition for preys and nesting areas observed by Gepp (2003) should only regard populations occurring in small villages and suburbs of larger towns and cities, without impact at a larger scale (see also Lukas et al., 2006). The possible ecological impact of the allochthon *S. caementarium* over the native *Sceliphron* species has been first discussed by Piek (1986), outlining that this alien species could replace the native species, namely referring to *S. spirifex*. Campadelli et al. (1999) provided evidence on a case of apparent replacing of the native species *S. destillatorium* in Emilia-Romagna, estimating this over a period of only ten years. Probably, it is

not possible to predict the replacement of native species in the case of coexistence with alien ones, due to local ecological features, e.g., availability of nesting places and prey abundance. For instance, *S. curvatum* in Sicily has been observed in semi-natural habitat, differently than observed in other European countries. Thus, whether the presence of alien *Sceliphron* in Sicily represents a true threat for the native species still remains under great uncertainty although this matter deserves further monitoring acts over a significant period to collect significant data on the distribution and the basic ecological traits.

FAUNISTIC SUMMARY ON THE NATIVE *SCELIPHRON* FROM SICILY

Although species of the genus *Sceliphron* in Italy are not of great faunistic interest, due to its prone to colonize a wide range of habitat, including those urban, in the following we provide a faunistic appendix with data on native *Sceliphron* from Sicily recorded in literature (including social forum of amateurs) along with material preserved in our collections. This is mainly to provide a basic framework to monitor their distribution and to relate it, in the near future, with those of alien species. Despite these species seems to be widespread and common in Sicily, the results clearly show that there are only few records and that the Regional distribution still remains poorly known.

Sceliphron destillatorium (Illiger, 1807)

DATA FROM LITERATURE. Sichel, 1860 (Sicilia); De Stefani Perez, 1881 (Sciaccia: torrente Carabolace); De Stefani Perez, 1883, 1889, 1895 (Sicilia); Giordani Soika, 1944 (Messina; Falcone; Fiumetorto; Agrigento); Pagliano, 1985 (Scicli); Pagliano, 1990 (Pizzenti); Gayubo et al., 1991 (Levanzo; Maraone); Negrisol, 1995 (Sicilia); Schmid-Egger, 2003 (Castellammare; Ragusa); Pagliano & Negrisol, 2005 (Sicilia).

DATA FROM WEB SOURCES. Trapani province: Fulgatore, 15.VI.2012, M. Ferrante (F.E.I.). Ragusa province: Modica, VII.2009, V. Risoldi (F.E.I.).

EXAMINED MATERIAL. Messina province: 1 female, Aeolian Islands, Panarea, 18.VI.1967, Sulfaro leg.; 1 male, Montalbano Elicona, Monte Polverello, 1257 m a.s.l., 37°58'48."N/ 14°57'35."E,



Figures 1, 2. Alien *Sceliphron* recorded from Sicily. Fig. 1: *S. caementarium*; Fig. 2: *S. curvatum*. Photos by Turrisi G.F.

11.VII.2015, on *Thapsia* sp., G. Altadonna leg. Catania province: 1 female, Piana di Catania, Contrada Primosole, 11.VI.1996, on flowers of *Cachrys sicula* L., G.F. Turrisi leg.; 1 male, 1 female, Iblei, Vizzini, Fiume Vizzini, Contrada Rubalà, 320 m a.s.l., 29.V.1999, G.F. Turrisi leg. Syracuse province: 1 female, Iblei, Noto, Testa dell'Acqua, 1.VII.1993, G.F. Turrisi leg.

REMARKS. Newly recorded for Aeolian Islands (Panarea), already known for other circumsicilian islands (Levanzo, Maraone). Interesting biological information on this species in Italy have been provided by Campadelli & Pagliano (1987).

Sceliphron madraspatanum tubifex (Latreille, 1809)

DATA FROM LITERATURE. Sichel, 1860 (Sicilia); De Stefani Perez, 1881 (Sciaccà: torrente Carabol-lace); De Stefani Perez, 1886 (Palermo: Monti di Renda); De Stefani Perez, 1889, 1895 (Sicilia); De Stefani Perez, 1894 (Trapani: Santa Ninfa); Giordani Soika, 1944 (Falcone); Pagliano, 1985 (Cefalù); Negrisolò, 1995 (Sicilia); Schmid-Egger, 2003 (Castellammare); Pagliano & Negrisolò, 2005 (Sicilia); Pagliano, 2008 (Sicilia).

DATA FROM WEB SOURCES. Trapani province: Marausa, 27.VII.2013, L. Barraco (F.E.I.).

EXAMINED MATERIAL. Catania province: 1 male, Fiume Alcantara, Calatabiano, 13.VIII.1991, G.F. Turrisi leg. Caltanissetta province: 1 male, Gela, Biviere di Gela, 16-17.VII.2005, G.F. Turrisi leg.; 1 male, same locality, 27.V.2006, G.F. Turrisi leg.

Sceliphron spirifex (Linnaeus, 1758)

DATA FROM LITERATURE. Riggio & De Stefani Perez, 1888 (Ustica); De Stefani Perez, 1881 (Sciaccà: torrente Carabol-lace); De Stefani Perez, 1883, 1889, 1895 (Sicilia); De Stefani Perez, 1886 (Palermo: Monti di Renda); De Stefani Perez, 1894 (Trapani: Santa Ninfa); Giordani Soika, 1944 (Messina; Falcone; Agrigento); Pagliano, 1985 (Scicli; Cefalù; Marsala; Palermo); Pagliano, 1990 (isola di Lampedusa); Negrisolò, 1995 (Sicilia); Pagliano & Scaramozzino, 1995 (Lampedusa); Schmid-Egger, 2003 (Avola); Pagliano & Negrisolò, 2005 (Sicilia).

DATA FROM WEB SOURCES. Trapani province: Marausa, 15.VIII.2009, L. Barraco (F.E.I.).

EXAMINED MATERIAL. Messina province: 1 female, Aeolian Islands, Vulcano Island, Piano, 330 m a.s.l., 6.VII.2012, G. Altadonna leg.; 1 female, Messina, Tremestieri, 30 m a.s.l., 38°08'33.0"N 15°31'20.6"E, 3.III.2015, dead specimen, G. Altadonna leg.. Catania province: 1 male, Fiume Alcantara, Calatabiano, 13.VIII.1991, G.F. Turrisi leg.; 1 male, same locality, 4.VI.1999, G.F. Turrisi leg.; 1 male, Etna, Pedara, 700 m a.s.l., VII.2006, A. Vaccaro leg.; 1 female, Etna, Gravina di Catania, 350 m a.s.l., 4.XI.1991, C. Arena leg.; 1 male, Etna, Tremestieri Etneo, 350 m. a.s.l., 20.X.1991, G.F. Turrisi leg.; 1 female, same locality, 12.IX.1995, E. Turrisi leg.; 1 female, San Gregorio di Catania, 200 m a.s.l., 28.IX.1990, S. Bella leg.; 1 female, Piana di Catania, Contrada Primosole, 16.VII.2002, A. Messina leg.; 1 male, same locality, 19.VIII.2009, on leaves of *Punica granatum* L., G.F. Turrisi leg.. Syracuse province: 1 male, Iblei, Pachino, VII.1994, C. Bella leg. Caltanissetta province: 4 males, Gela, Biviere di Gela, 16-17.VII.2005, G.F. Turrisi leg.; 1 male, same locality, 27.V.2006, G.F. Turrisi leg.

REMARKS. Newly recorded for Aeolian Islands (Vulcano), already known for other circumsicilian islands (Ustica and Lampedusa).

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First records of breeding *Sympecma paedisca* (Brauer, 1877) (Odonata Lestidae) in Italy

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ABSTRACT

Oviposition in Italian populations of *Sympecma paedisca* (Brauer, 1877) (Odonata Lestidae) was observed for the first time. This species is listed as Endangered (EN) in the Mediterranean Basin and as Critically Endangered (CR) in Italy. Several ovipositing tandems were observed for the years 2014, 2015 and 2016, from the 17th of May to the 10th of June, in the “Riserva Naturale Orientata della Baraggia di Candelo” (=Heathlands Oriented Natural Reserve) (North Piedmont), a protected area and a military zone too, in a pond at the edge of the heathland. Oviposition substrates are vertical living *Juncus effusus* L. stems, preferably the isolated ones or those on the external side of the tufts, rather than inside them; eggs are laid about 20 to 50 cm above the water level. In the heathland, around the breeding site, tens of adults were seen every autumn and winter, also in December and January sunny days. Reproductive *S. paedisca* were also occasionally observed in other two localities, namely the lake of Viverone and the “Riserva Naturale Orientata Palude di Casalbeltrame” (=Casalbeltrame Fen Oriented Natural Reserve). Notes on breeding behaviour and a description of both breeding and overwintering area of *S. paedisca* are provided, since knowledge of its breeding and overwintering sites is needed to ensure their protection and therefore the conservation of Italian populations of this damselfly.

KEY WORDS

Sympecma; Odonata; Heathland; Italy; Military area.

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INTRODUCTION

The Siberian Winter Damselfly *Sympecma paedisca* (Brauer, 1877) (Odonata Lestidae), sometimes reported as *S. braueri* or *S. annulata braueri* (Bianchi, 1904) is a lestid dragonfly ranging from Western Europe through central Asia to Japan. In Europe it is still common in the north-east (Poland, Baltic States, southern Finland), rare and very localized elsewhere. It is supposedly extinct in France and parts of Germany (Jurzitza, 1961; Bilek, 1964; Boudot et al., 2009), as well as in the north-east of

Italy (Canovi et al., 2014; Riservato et al., 2014a), because of habitat loss due to human influence (Riservato et al., 2009; Ceballos et al., 2015). In Italy recent records are mainly for north-eastern Piedmont (Sindaco et al., 2003; Boano et al., 2007; Battisti, 2014; Riservato et al., 2014b), with a recent confirmation for Lombardy (Canovi et al., 2014). These southern European populations, the only ones still extant south of the Alps, are highly disjunct from the main range. *Sympecma paedisca* is accordingly listed as Endangered (EN) in the Mediterranean Basin and as Critically Endangered

(CR) in Italy (Riservato, 2009, 2014b). It is also included in Annex IV of the Habitat Directive 92/43/CEE.

Despite of being *S. paedisca* in Piedmont regularly occurring in several sites, until recent years no reproductive biotope was actually known (Riservato et al., 2014a).

Below is reported the first record of breeding *S. paedisca* in Italy.

RESULTS AND DISCUSSION

A potentially suitable site in Piedmont (NW Italy), namely the SCI IT1130003 “Baraggia di Candelo”, within the “Riserva Naturale Orientata delle Baragge” (= Heathlands Oriented Natural Reserve) (RNOB), was investigated. The RNOB is a natural reserve and a military area too. It is a plateau of about 1600 ha, characterized by moorland open areas surrounded by oak forests, inside a traditional agricultural landscape matrix. The SCI IT1130003 “Baraggia di Candelo” is included in the “Natura 2000” network, because of the presence of some European priority habitats, such as: “European dry heaths”, “*Molinia* meadows (*Molinion caeruleae*)”

and “Depressions on peat substrates of the *Rhynchosporion*”(Sindaco et al., 2003). Here a large population of *S. paedisca* occurs, with tens of adults seen from September up to the following spring. Adults overwinter inside the heathland and the *Molinia* grassland and are active also in December and January sunny days (Battisti, 2014; Battisti & Soldato, 2014). Tens of waterbodies are scattered through the heathland, ranging from fairly large permanent ponds to smallest temporary bogs; some of them are man-made, to ensure cattle watering. Researches particularly focused on a permanent, spring-fed pond at the very edge of the heathland, close to the cultivated area (Fig. 1). It is located about 12 km southeast of Biella, 240 m a.s.l., not far from the village of Villanova Biellese. It is partly bordered by more or less dense *Juncus* formations, with a sector of reed belt along the shore with deeper water. The side with shallower water is surrounded by bare soil or very short grass, because of the use of the pond for grazing and watering by cattle. The site is outside the Natura2000 SCI, yet it is included in the RNOB and close to the boundary of the military area.

On 1.VI.2014, several (> 20) ovipositing tandems (Fig. 4), as well as very few copulae, were

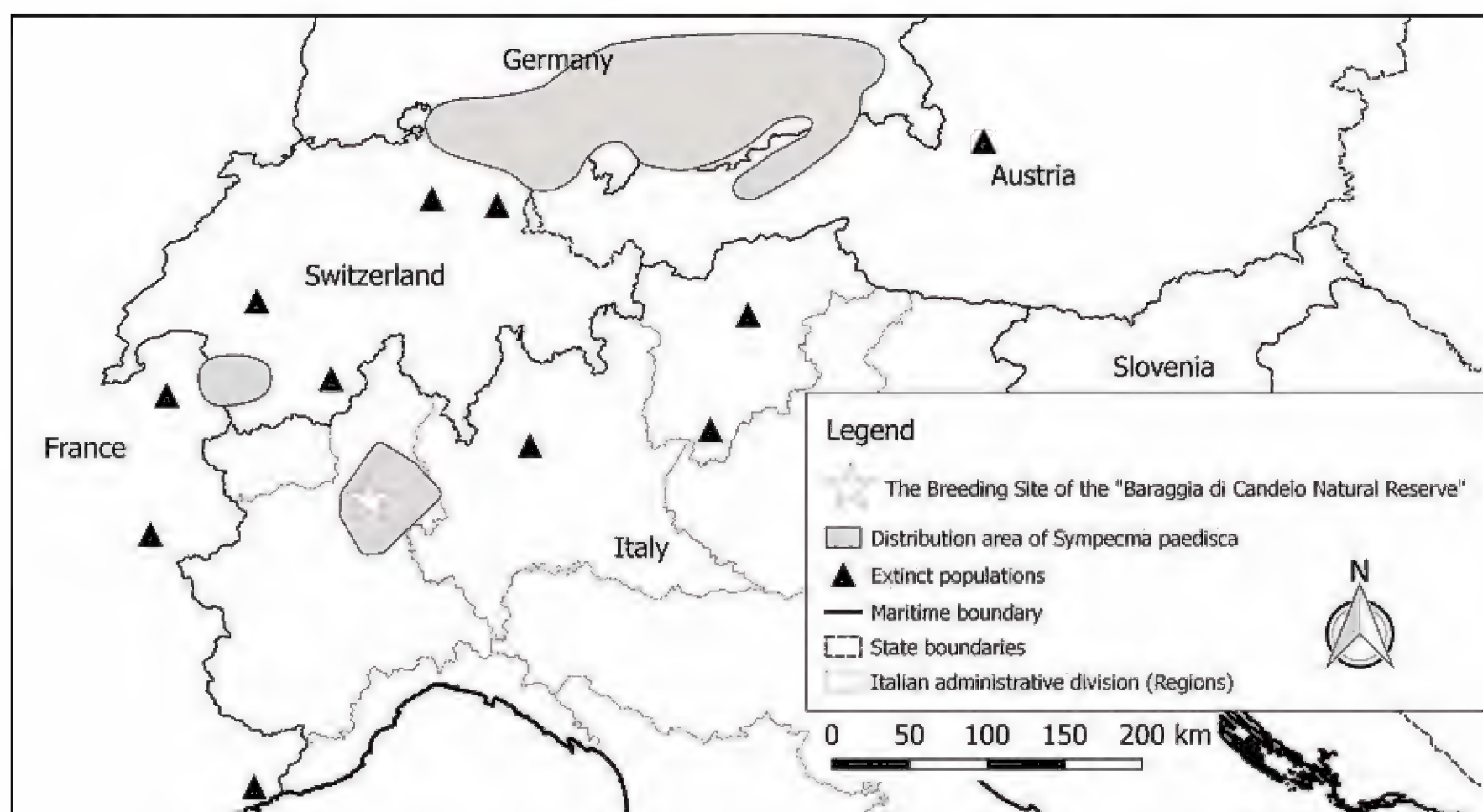


Figure 1. Occurrence of *Sympecma paedisca* in Italy and in adjacent areas, after Dijkstra (2006), changed according to Riservato et al. (2014) and personal observations for Italy and according to Boudot & Kalkman (2015) and to Siesa (2017) for adjacent states.



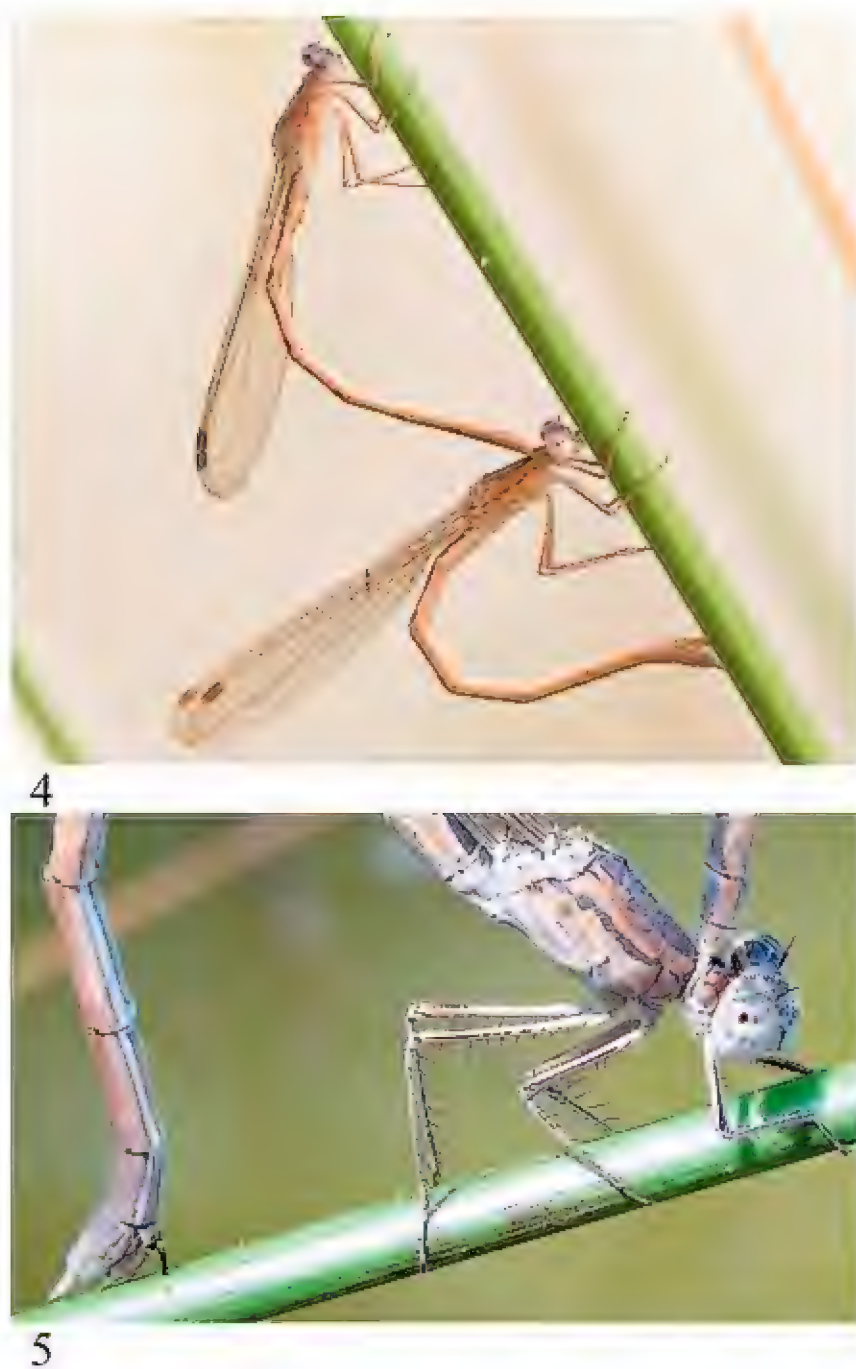
Figures 1–3. SCI “Baraggia di Candelo”, Riserva Naturale Orientata delle Baragge (= Heathlands Oriented Natural Reserve) (Biella, NW Italy). Fig. 1: the monitored pond, breeding biotope of *Sympecma paedisca* (by M. Pavese). Fig. 2: *Juncus effusus* L. tuft, oviposition site (by A. Battisti). Fig. 3: *Molinia* meadow, maturation and overwintering biotope; in the foreground a small temporary bog (by A. Battisti).

observed; decreasing numbers of ovipositing tandems (up to 3) were noticed until 7.VI.2014. Observations were repeated on 17–18.V.2015, 28.V.2016 and 10.VI.2016, with smaller numbers of tandems (< 10 each time) and a unique unaccompanied ovipositing female. The oviposition substrates are, almost always, living *Juncus effusus* L. stems (Fig. 2), where the eggs are laid about 20–50 cm above the water level (Fig. 3). Only one tandem was seen ovipositing, or at least attempting to do so, in a dead standing stem. Oviposition seems to take place preferably on isolated stems of *Juncus* or on the external ones of the tufts, rather than inside them. Despite of repeated visits in the weeks following each oviposition record, no emerging adults, exuviae or larvae could ever be noticed.

In Italy, at present, this is the only site, at least in recent times, where *S. paedisca* breeding was

regularly observed over more years. We report two further recent records of ovipositing *S. paedisca*. One unaccompanied female was photographed when laying eggs in a stem, most likely of *Carex* or *Phragmites*, at the Lake of Viverone (Azeglio, Province of Turin) on 24.V.2015 (post by Gianni Cattin, “willy98”, on Forum Natura Mediterraneo: http://www.naturamediterraneo.com/forum/topic.asp?TOPIC_ID=252132, determination by MP, “gomphus”). This site is included in the Natura2000 network as SCI/SPA IT1110020 “Lago di Viverone”, and is located about 15 km from the breeding site of the RNOB. Here the species is not common; there are only few records of non-breeding adults before 2000 (Riservato et al., 2014a), and some recent records (R. Sindaco pers. comm.).

E. Subrero (pers. comm.) observed few tandems at the “Riserva Naturale Orientata Palude di Casal-



Figures 4, 5. Oviposition of *Sympecma paedisca*. Fig. 4: ovipositing tandem (by A. Battisti). Fig. 5: detail of oviposition, showing ovipositor blade piercing the stem, at the end of a row of previous oviposition holes (by M. Pavesi).

beltrame” (= Casalbeltrame Fen Oriented Natural Reserve) (Province of Novara) on 7.V.2013, as well as very few copulae and one ovipositing tandem, laying eggs into living stems of unspecified aquatic plants, on 11.VI.2014. This site is included in the Natura2000 network as SCI IT1150003 “Palude di Casalbeltrame” and is located about 30 km from the breeding site of the RNOB.

During a research in the Netherlands, marked individuals of *S. paedisca* were observed 40 km away from the breeding sites (Ruiter et al., 2007). It is possible that dispersal of vagrant individuals outside the regular breeding area may occasionally result in oviposition in more or less unsuitable biotopes, with possible temporary breeding sites, yet without any permanently established populations. We therefore wait for further records, before con-

firming the other two sites as permanent breeding ones. Conversely, the high winter presence of adults throughout the heathland of the RNOB, the presence of several breeding individuals (copulae and ovipositing tandems) for three consecutive years and the variety of potentially suitable habitats, such as ponds and moorlands, most of them not yet adequately investigated, argue for being this place of greatest importance as both a breeding and an overwintering area.

Ruiter et al. (2007) hypothesize that *S. paedisca* can move many kilometres from the breeding sites to the wintering ones, selecting heathlands to overwinter; we can add *Molinia* meadows (Fig. 3) and other types of high grass meadows as overwintering areas too, regardless of the presence of water in the winter time. On the other hand, some observations (Canovi et al., 2014) suggest that, as for studied populations, vagility in *S. paedisca* may be by far lesser than in the congeneric and syntopic *S. fusca* (Vander Linden, 1820). Mark/recapture programs will be needed to solve the question.

Reproductive behaviour of *S. paedisca*, from our observations, seems to differ from that of *S. fusca*. The latter is reported to mate and oviposit early in spring, already in March, and to lay eggs in floating debris at the water surface. All tandems of *S. paedisca*, as well as the very few unaccompanied females, were seen to lay eggs into vertical, all but one living, stems, well over water surface. Moreover, recorded oviposition time is distinctly later. On 25.IV.2009, 29.III.2014, 20.III.2015, 27.III.2015 and 25.IV.2015, within 6 km of the studied pond, several *S. paedisca* were found, yet no sign of reproductive behaviour was noticed at, or close to, the small waterbodies here existing. On 29.IV.2017, at the same pond, 2 males were seen at the water edge, 2 males and 3 females in the close surroundings, again with no sign of reproductive behaviour. All the latter males, and those observed on 25.IV.2009, were seemingly not fully mature, having only a small area on the top of the eyes turned blue, instead of the whole dorsal face. The earlier observation of oviposition at the Baraggia di Candelo is on 17.V, the later on 10.VI (at Casalbeltrame on 11.VI). It is to be stressed that no emerging adults, exuviae or larvae were noticed in the weeks following each oviposition record, possibly because of a wrong estimation of life cycle time. Only one fresh, recently emerged individual was

recorded on 16.VIII.2012. The first maturing adults, in heathland and *Molinia* meadows, were noticed already away from water at the end of August.

Sympecma paedisca oviposition in vertical substrates is also reported for eastern Kazakhstan (Reinhardt & Gerighausen, 2001). Manger (2007) conversely observed in the Netherlands oviposition in floating debris, starting from 14.IV; the same author also states he could observe at the same site no ovipositing tandems nor mating pairs of the co-occurring *S. fusca*, despite the presence of mature individuals, thus seeming to confirm a non-synchronous oviposition of the two species.

NW Italian population of *S. paedisca* is the only one still extant south of the Alps and of the parallel 45°48'N. Information on breeding behaviour and a description of a both breeding and overwintering area of *S. paedisca* herewith provided are a first step towards a better knowledge of its ecological requirements and life cycle, as well as a more extensive detection of breeding and overwintering sites, in order to ensure adequate protection, all of them essential for the conservation of this southern population. We suppose that several other reproductive sites, besides those above described, may exist in NW Italy, and that lack of information may be largely due to a wrong estimation of the oviposition period, this resulting in surveys carried out in inappropriate periods. Future investigations throughout the known Italian range will hopefully fill the gaps.

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Contributions to the malacology of Malta, III: First record of *Planorbella duryi* (Wetherby, 1879) (Gastropoda Planorbidae) for Comino

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ABSTRACT

The freshwater and allochthonous species *Planorbella duryi* (Wetherby, 1879) (Gastropoda Planorbidae) (= *Helisoma duryi* Wetherby, 1879) is reported from the island of Comino (Maltese archipelago). This is the first record of a freshwater species and also of an allochthonous species for the third largest island of the Maltese archipelago.

KEY WORDS

Allochthonous species; *Planorbella*; Maltese archipelago; Comino.

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INTRODUCTION

Alien species of non-marine molluscs in the Maltese islands have already been treated in detail in various studies (Giusti et al., 1995; Beckmann, 2003; Mifsud et al., 2003; Barbara & Schembri, 2008; Cilia, 2009; Cilia et al., 2012a). Many of these species were noted to be restricted to anthropogenic habitats, but the potentially invasive character of some species should not be underestimated (Barbara & Schembri, 2008; Cilia et al., 2012a; Cilia, 2014).

Herein, the freshwater species *Planorbella duryi* (Wetherby, 1879) (Gastropoda Planorbidae) (= *Helisoma duryi*) is reported from the island of Comino (= Kemmuna) for the first time. A long-term previous study about non-marine molluscs of this island and others, carried out from 1998 to 2012, did not reveal any specimens of any freshwater species (Cilia et al., 2012b). Therefore, this is the first record of a freshwater species and also the first record of an allochthonous species for the third largest island of the Maltese archipelago.

METHODS AND RESULTS

A small population of *P. duryi* consisting of several specimens in various stages of growth was discovered and studied in situ on the 13th of May of 2017 in a water reservoir, presumably used to store water for use in the little arable land in the same valley, close to Santa Marija Bay (36°00'52"N 14°20'12"E). Some live individuals were collected for further observation in a laboratory setting.

In addition to the mollusc population, various larvae of Ephemeroptera and Odonata were observed, as well as some individuals of an unidentified freshwater fish. A carpet of chlorophytes covered most of the available surface, but the water itself showed very little turbidity. Another similar reservoir in the vicinity (36°00'41"N, 14°20'29"E) did not seem to contain any specimens of *P. duryi*.

One of the adult specimens collected for further study laid a batch of 20 eggs, encased in a white gelatinous membrane, using the plastic container as a substrate. On their first day, these eggs were a

very pale yellow-brown with a miniscule black nucleus at their periphery.

DISCUSSION AND CONCLUSIONS

All previous records and mentions of *P. duryi* in the Maltese islands (Beckmann, 1987; Beckmann, 1988; Giusti et al., 1995; Cachia, 1999; Mifsud et al., 2003) and in Italy (Mastrantuono, 1990; Mienis, 2004; Zettler & Richard, 2003 [as *Planorbella anceps* (Menke, 1830); the figures in this paper also indicate a misidentification]; Alexandrowicz, 2003; Cianfanelli et al., 2007; Reitano et al., 2007; Sparacio et al., 2017) seem to indicate that the species is subject to passive dispersal with ornamental freshwater plants and with the introduction of freshwater fish, and therefore mostly restricted to private aquariums, fountains, greenhouses and reservoirs, with the occasional foray into natural ecosystems. Such localized, semi-naturalized populations are highly prone to population explosions and extinctions. Notably, a survey of species in Lago Albano (Lazio, Italy) in 1950 omits the species (Stella, 1951), with the first living examples being discovered in 1986 (Mastrantuono, 1990). By 2008, no more living specimens were to be found occurring in this locality (Mastrantuono et al., 2011). Likewise, a population in a small stream in Wiedil-Luq (Rabat, Malta) became extinct around 1988 due to drying up of the stream. The present author can also recall sizeable populations in Maltese public and private gardens in the 1990s (Romeo Romano Gardens, Santa Venera; Vincenzo Bugeja Institute, Santa Venera) that have since become extinct.

Other allochthonous planorbids, namely *Ferrissia californica* (Rowell, 1863), reported as *F. fragilis* (Tryon, 1863), that is a junior synonym of *F. californica* (see Christensen, 2016), a placement also confirmed through COI and 16S sequencing (F. Marrone, *in litteris*), and *Planorbarius corneus* (Linnaeus, 1758) have been previously recorded from the Maltese islands (Mifsud et al., 2003; Cilia, 2009). Of these, only *Pl. corneus* has a shell that is superficially similar to that of *P. duryi*, though its adult size is usually much bigger, with a more corrugated periostracum. More reliable methods of distinguishing *P. duryi* from *Pl. corneus* are a slight angulation on the dorsal surface of the whorl and

the dilated, auriform outline of the peristome in *P. duryi*. The congeneric *P. anceps* (Menke, 1830), recorded from natural habitats in River Frigido (Henrard, 1968) [maybe also case of mistaken identity with *P. duryi* (cf. Cianfanelli et al., 2007; Marrone & Naselli-Flores, 2015)] and Lake Prespa (Eröss et al., 2005), has a prominently flared peristome not observed in any of the specimens studied for this research.

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